THE JOURNAL
OF
CANCER RESEARCH

EDITED BY
WILLIAM H. WOGLOM

ASSOCIATE EDITOR
FREDERICK PRIME

VOLUME IV

BALTIMORE, MD.
1919

CONTENTS

Number 1, January, 1919

Number 2, April, 1919

Number 3, July, 1919

Number 4, October, 1919

VIRULENCE OR ADAPTATION?[1]

WM. H. WOGLOM

From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director

Received for publication, December 22, 1917

REVIEW

Although all observers agree that once a mouse tumor has been transplanted it generally yields an increasing number of daughter tumors upon protracted cultivation, it is not yet known whether this is to be referred to an increase in "virulence" on the part of its cells, or to an augmentation in their power to adapt themselves to each new host successively. The former hypothesis has been supported by Ehrlich (1) and Apolant (2), the latter by Bashford, Murray, Haaland, Bowen, and Cramer (3, 4, 5).

The English investigators urged great caution in comparing the growth of transplanted cancer cells with the cultivation of pathogenic microorganisms, and explained the higher percentage of success and the more rapid growth by an increase in the adaptability of the neoplastic cells to each new environment and an elimination of those elements less able to survive.

They observed frequently a rise of the transplantation outcome to a maximum, and a subsequent rapid diminution in the percentage of success, which in turn, was succeeded by an increase to the maximum. By choosing a suitable interval for the inoculation of tumors selected from series with from 90 to 100 per cent of takes, and especially by increasing the dose, the authors had been able to evade for a considerable number of

[1] A summary of this paper was presented at the Tenth Annual Meeting of the American Association for Cancer Research, held in New York on April 5. 1917. Cf. Jour. Cancer Research, 1917, ii, 503.

generations the diminution usually following each maximum. This outcome, however, was regarded by them as artificial, indicating neither an increased "virulence" of the tumor cells nor a uniform energy of growth. As a matter of fact, the "virulence" of the cells of any tumor fluctuates, they asserted, between negative and positive phases of growth energy.

Spontaneous tumors, they continued, vary considerably in the ease with which they can be propagated, and these differences had been regarded as degrees of "virulence;" but there exists a certain amount of evidence to show that such neoplasms fluctuate in growth energy in a manner similar to transplantable tumors, and any direct conclusion regarding the "virulence" of a spontaneous tumor drawn from one primary transplantation might accordingly be upset when the same growth gave an opposite result, i.e., when, after having recurred, it had been transplanted a second time.

The term "virulence" thus appeared to these authors an unsuitable one, to be used with so much reservation that it would be better discarded altogether.

Apolant distinguished sharply between two factors comprised in the conception of virulence—*transplantability*, measured by the number of daughter tumors, and *proliferative energy*, evaluated by the rate of growth. Transplantability can be stimulated, according to this investigator, by selecting at each inoculation the most rapidly growing tumor, but the proliferative energy can be hastened, apparently, only to a certain point specific for each strain. To these two qualities Ehrlich added a third, described provisionally as *exhaustive* or *ereptive;* he left open the question, however, whether it rests upon a maximum avidity of the tumor cells for nourishment, or upon some specific relationship toward the materials necessary for the growth of the neoplastic cells.

Apolant did not think that Bashford's hypothesis of adaptation sufficed to explain either the fact that tumors can be made to grow after a time in strange races of mice, or that it is possible to achieve an increase in "virulence." With Ehrlich, he believed that a tumor cell must have a number of potential rudi-

ments besides the actual nutriceptors suited to the food offered by the affected animal. If the growth be transplanted to another host, three occurrences are possible: either the new soil will afford the same nourishment as the old, and the tumor can accordingly grow; or the host will proffer food that none of the nutriceptors is able to grasp, in which case transplantation will be unsuccessful; or, finally, there may be tendered nutriment not exactly like the old, but for which there are present at least the potential rudiments of nutriceptors. In such a case, those cells will die in which the development of potential nutriceptors has not occurred with sufficient promptitude; but the elements which have been stimulated by hunger to produce fresh nutriceptors will be able to survive in the new host, and, furthermore, their proliferation through succeeding generations will be assured, since the newly acquired properties are inheritable.

This hypothesis Apolant regarded as entirely sufficient to explain the sudden changes in growth energy so frequently observed when neoplasms are introduced into strange races, as well as the increase in virulence so often exhibited by tumor cells.

To Haaland (6), also, Ehrlich's explanation appeared eminently suitable. Nevertheless, he regarded the "virulence" as a relative rather than an absolute quantity, since it varies enormously according to the strain of mice inoculated with the tumor in question; thus a new growth so "virulent" as to proliferate in nearly 100 per cent of mice of one strain, may very well prove almost incapable of growth in another.

Loeb (7) has on several occasions expressed the opinion that the increase in growth energy which comes to light after the transplantation of spontaneous tumors, is dependent to a great extent upon the mechanical stimulation of their cells through the process of transplantation.

If our main interest in cancer were reserved for the growth of transplantable tumors, a choice between the terms *virulence* and *adaptation* would be a doctrinaire question indeed. But investigation of the spontaneous tumors of mice will in all probability furnish a number of conclusions that can be transferred to human

neoplasms, which are, after all, the ultimate goal of inquiry, and the question whether or not tumors of the mouse mamma do differ in "virulence" may thus prove to be of really practical importance. But the word *virulence*, with all its tacit suggestion of some analogy between the tumors and the infectious diseases, is an unsuitable one, for which should be substituted some such purely descriptive and noncommittal expression as *proliferative vigor* or *growth energy*.

The evidence already collected appears to suggest that both proliferative energy and adaptation are concerned in the growth of propagable neoplasms. For example, it is well known that mouse tumors exhibit periodical fluctuations in the number of takes from generation to generation, even when they are transplanted into mice of the same strain; and since it is hardly likely that, having once become adapted to a soil, they should lose and regain their adaptability time after time, the most reasonable way of explaining this phenomenon is to refer it to variations in their proliferative energy.

Again, Murray (8) discovered a somewhat similar variability in spontaneous tumors. The percentage of successful grafts obtained by transplanting recurrences of such neoplasms was often entirely different from that following inoculation of the primary tumor; and he found, furthermore, that the behaviour of the spontaneous growth in the animal primarily affected might be almost exactly paralleled by that of the same tumor proliferating in normal animals after transplantation. Rapid recurrence of a primary tumor coincided with an initial rapid proliferation of the transplanted growths, while a long period of quiescence after a second operation was reflected in the transplantation series, where almost every nodule showed a corresponding cessation of growth, and some diminished in size. A second recurrence and renewed rapid growth of the primary tumor was practically synchronous with a similar behavior in the transplanted growths. Russell (9), also, has observed a discrepancy between the inoculation percentage of a tumor and its recurrence, and, like Murray, has ascribed it to cyclic changes in the life of the tumor cell.

All these observations suggest the occurrence of variations in the proliferative energy of the cancer cell, but they do not touch the question of adaptation to a new host. Other evidence, however, seems to show that tumors are able gradually to accustom themselves to nutrient material that is at first more or less foreign, for lying beneath the cyclic variations there is, after all, like a sort of ground-tone, a greater tendency for neoplasms to succeed on removal to another host after they have been under cultivation for a time. That this is true may be safely assumed from Bashford's growth curves (10), of which one (his fig. 6) is here reproduced as an example of the general upward trend accompanying continued propagation (fig. 1). Further evidence of the factor of adaptation is to be found in the observation, substantiated by so many observers, that a spontaneous tumor grows

FIG. 1. GRADUAL RISE OF INOCULATION PERCENTAGE
Redrawn from Bashford's figure 6

better when reinoculated into the animal in which it arose, than it does in any other animal; for since there is no reason to suppose that its growth energy varies in the two cases, it must be assumed that it proliferates more successfully in the animal to which it is native because it need make no effort to adapt itself to the soil.

EXPERIMENTS

In order to analyse these two factors, adaptation and growth energy, the following experiments were undertaken; but it is realized, of course, that there may be still other factors involved. The question is: Do some spontaneous mouse tumors succeed upon first transplantation more easily than others because their parenchyma is more plastic as concerns its power to adapt itself to a new host, or merely because they happened to be transplanted at a time when their growth energy was high?

Multiple mammary carcinomata are of frequent occurrence in mice, some of which have as many as seven of these growths.

Suppose now that several spontaneous neoplasms (X, Y, etc.) from the same mouse be inoculated into a series of normal mice from the same source. Since these carcinomata are all composed of tissue from one and the same animal, they should be equally able (or unable) to accustom themselves to an unfamiliar soil; in other words, after having been transplanted, all should produce the same number of daughter tumors, or nearly so, if adaptation were the sole factor controlling the inoculation outcome. If, on the other hand, the number of daughter tumors be conditioned solely by the growth energy of a spontaneous neoplasm at the time of its transplantation, then it is conceivable that primary tumors from the same mouse would vary in their inoculation percentages, and X might give a higher proportion of success than Y, for example.

In order to carry out such an experiment, two assumptions must be made:—that mice from the same dealer offer, in the long run, a soil approximately similar; and that the multiple spontaneous tumors of mice are not metastatic. In regard to the first, it may be asserted that where sufficiently large numbers of animals are employed, individual differences tend to disappear. As for the second, both Gierke (11) and Haaland (12) are of the opinion that a tendency to primary multiplicity exists in the mouse, the latter author advancing, among other reasons, the invariable location of these additional tumors in the mammary apparatus and the wide distances which often separate them, in support of his contention that they are not metastatic.[2] Murray (13), it is true leaves the question open; but his article was written several years before that of Haaland, and thus at a time when the number of spontaneous tumors that had come under observation at the London institute was not so great as Haaland could command. Hence it would be unfair to advance this as his mature opinion.

[2] Since this was written, an article by Fischer (Medicinsk Revue, 1916) has come to my attention. His experiments, conducted in Haaland's laboratory, show that India ink injected into one mouse mamma never appears in another one. Fischer concludes that cancer cells, therefore, probably do not travel from one mamma to another, and that in all probability multiple tumors in mice are independent (not metastatic) growths.

Nevertheless, in order to reduce to a minimum the chances of error in the following experiments, only those tumors were chosen for transplantation which occupied diagonal positions (e.g., right axilla and left groin) and were thus as remote from one another as they could be, except of course, where more than two neoplasms were used; in such cases this precaution obviously could not be observed.

The tumors from any one mouse were invariably inoculated at one sitting, and by one man, in order to guard against personal variations in technic. This is true in every case except that of mouse $\frac{255}{0}$, which had five tumors; in order that the implantation might be finished within a time roughly comparable to that necessary in the case of other series, five men made the inoculations. It is of interest to note, in passing, that the variations in the case of these five tumors were not so great as occurred in some other series where one man did all the inoculations.

Other precautions were observed to keep the conditions as uniform as possible. Thus, during the implantation of the first tumor the second was kept in the ice-box; 150 mice were engrafted with each tumor in almost every case, in order to eliminate, as far as possible, the fallacies inherent in small numbers; finally, the animals employed for implantation of the growths from any one spontaneously affected mouse were always derived from the same dealer, were of approximately the same age, and were kept after inoculation in the same room and on the same diet. The daughter tumors were charted one month after their implantation and every week thereafter for eight weeks, experience having shown that practically no growths appeared after the lapse of three months. In computing the outcome of an inoculation, it is often difficult to decide whether a mouse with an insignificant stationary or receding tumor is to be included with the positive or the negative animals. Since microscopic examination showed that even the very smallest nodules were composed of healthy tissue, unless undergoing retrogression, it was determined to regard every animal as positive in which any tumor, however small, had appeared, and this even though the graft remained station-

ary or receded; for it was merely the question whether or not the cells of the neoplasm were able to commence growth in another animal that was under investigation, the question of their ultimate success being confused by other factors unconnected with this problem. Nevertheless, the figures thus gained were tested by comparison with others obtained through counting only the progressively growing tumors, when it was found that, although the actual percentages in the former cases were of course higher, the comparative figures remained the same. In other words, of any two or more spontaneous growths from the same mouse, that one which gave the highest percentage of all sorts of daughter tumors (progressively growing, stationary, and receding) also gave the highest percentage of progressively growing ones. Hence the figures represent as close an approach to accuracy as it is possible to obtain in a biological experiment.

Where all the mice in a box were dead at the end of the eighth week (which was not often the case), those remaining alive at the seventh, sixth, fifth, or fourth were counted. But no negative mice dying before the fourth charting were included, though positives which died before this time were.

As the following figures show, 101 spontaneous neoplasms (of which 2 were recurrences) from 44 mice were employed in this investigation. The smallest number from any one animal was 2, and the largest 5. All were carcinomata, except in the case of two mice. In $\frac{310}{0}$, tumor Y was a carcinosarcoma; in $\frac{315}{0}$, tumor Y produced sarcoma in a growth of the first generation, although the slide from the spontaneous neoplasm contained nothing but pure carcinoma. In both cases, however, the differences were much less than often appeared after the transplantation of two carcinomata from the same mouse.

The mere number of daughter tumors may be taken as an index of adaptation and growth energy with comparative safety, without troubling in each individual case to analyze this into the number of receding, stationary, and growing nodules; for it appears to be a general rule that where the percentage of takes is low, most of the tumors will be of insignificant size, and when it is high many of them will grow progressively.

MOUSE	TUMOR	RESULT	SIZE OF TUMORS AND CHARACTER OF THEIR GROWTH	DIFFERENCE
112/0	W	$\frac{0}{93} = 0\%$		Less than 10%
	Y	$\frac{0}{85} = 0\%$		
159/0	X	$\frac{0}{137} = 0\%$		Less than 10%
	Y	$\frac{2}{106} = 2\%$	Pinhead to pea-size. Receding	
145/0	T	$\frac{2}{127} = 2\%$	1 pinhead receding; 1 pea very slowly growing	17%
	U	$\frac{22}{117} = 19\%$	Pinhead to hickory nut. Progressive growth	
158/0	X	$\frac{0}{82} = 0\%$		Less than 10%
	Y	$\frac{1}{69} = 1\%$	Pea-size. Receding	
	X (recurrence)	$\frac{3}{60} = 5\%$	Small pea. Stationary or receding	Less than 10%
	Y (recurrence)	$\frac{5}{49} = 10\%$	Small pea. Stationary or receding	
182/0	W	$\frac{0}{66} = 0\%$		30%
	X	$\frac{43}{141} = 30\%$	Pinhead to hickory nut. Progressive	
	Y	$\frac{4}{79} = 5\%$	Pinhead to pea. Receding	
202/0	X	$\frac{1}{82} = 1\%$	Pea-size. Progressive	Less than 10%
	Y	$\frac{8}{106} = 7\%$	Hazelnut to walnut. Progressive	

MOUSE	TUMOR	RESULT	SIZE OF TUMORS AND CHARACTER OF THEIR GROWTH	DIFFERENCE
$\frac{206}{0}$	X	$\frac{4}{107} = 4\%$	Pinhead. Receding	Less than 10%
	Y	$\frac{7}{79} = 9\%$	Pinhead to pea. Receding	
$\frac{212}{0}$	X	$\frac{23}{91} = 25\%$	Pinhead to cherry-stone. Receding	Less than 10%
	Y	$\frac{29}{117} = 25\%$	Pinhead to hazelnut. Majority progressive	
$\frac{200}{0}$	W	$\frac{3}{108} = 3\%$	Pinhead to pea. Receding	17%
	X	$\frac{22}{109} = 20\%$	Pinhead to pea. Majority receding	
$\frac{234}{0}$	X	$\frac{0}{93} = 0\%$		Less than 10%
	Y	$\frac{6}{84} = 7\%$	Pinhead to pea. Receding	
$\frac{251}{0}$	X	$\frac{22}{94} = 23\%$	Pinhead to hazelnut. Majority stationary	11%
	Y	$\frac{8}{67} = 12\%$	Pinhead to pea. Receding	
$\frac{255}{0}$	T	$\frac{0}{38} = 0\%$		28%
	U	$\frac{7}{64} = 11\%$	Pinhead to small pea. Receding	
	W	$\frac{12}{43} = 28\%$	Pinhead to cherry-stone. Majority stationary	
	X	$\frac{0}{79} = 0\%$		
	Y	$\frac{5}{74} = 7\%$	Pinhead to pea. Receding	

MOUSE	TUMOR	RESULT	SIZE OF TUMORS AND CHARACTER OF THEIR GROWTH	DIFFERENCE
$\frac{275}{0}$	X	$\frac{0}{63} = 0\%$		25%
	Y	$\frac{16}{65} = 25\%$	Pinhead receding to hazelnut slowly progressive	
$\frac{315}{0}$	X	$\frac{0}{72} = 0\%$		20%
	Y	$\frac{14}{68} = 20\%$	Pea receding to walnut progressive	
$\frac{370}{0}$	X	$\frac{10}{86} = 12\%$	Pinhead. Stationary or receding	10%
	Y	$\frac{2}{81} = 2\%$	Pinhead. Receding	
$\frac{349}{0}$	X	$\frac{21}{77} = 27\%$	Majority pinhead to pea receding. Two progressive	15%
	Y	$\frac{9}{77} = 12\%$	Pinhead. Receding	
$\frac{375}{0}$	X	$\frac{4}{69} = 6\%$	Pinhead. Receding	Less than 10%
	Y	$\frac{0}{55} = 0\%$		
$\frac{396}{0}$	X	$\frac{3}{63} = 5\%$	Pinhead. Receding	Less than 10%
	Y	$\frac{0}{43} = 0\%$		
$\frac{394}{0}$	W	$\frac{18}{85} = 21\%$	Pinhead to pea. Receding	Less than 10%
	X	$\frac{17}{110} = 15\%$	Majority pinhead to pea receding	
	Y	$\frac{15}{95} = 16\%$	Pinhead to pea. Receding	

MOUSE	TUMOR	RESULT	SIZE OF TUMORS AND CHARACTER OF THEIR GROWTH	DIFFERENCE
$\frac{445}{0}$	X	$\frac{26}{57} = 46\%$	Pea to hickory nut. Progressive	11%
	Y	$\frac{37}{65} = 57\%$	Pea to walnut. Progressive	
$\frac{474}{0}$	X	$\frac{2}{74} = 3\%$	1 pea receding; 1 hazelnut progressive	49%
	Y	$\frac{48}{93} = 52\%$	Pea to hickory nut. Progressive	
$\frac{559}{0}$	X	$\frac{43}{88} = 49\%$	Pea to hickory nut. Progressive	Less than 10%
	Y	$\frac{45}{97} = 46\%$	Pea to hickory nut. Progressive	
$\frac{463}{0}$	X	$\frac{11}{73} = 15\%$	4 hazel to hickory nut	12%
	Y	$\frac{2}{59} = 3\%$	Pinhead	
$\frac{645}{0}$	X	$\frac{1}{46} = 2\%$	Pinhead	Less than 10%
	Y	$\frac{1}{33} = 3\%$	Pinhead	
$\frac{605}{0}$	X	$\frac{0}{47} = 0\%$		41%
	Y	$\frac{24}{58} = 41\%$	Majority progressively growing, walnut size	
$\frac{809}{0}$	X	$\frac{34}{97} = 35\%$	Pinhead to hickory nut. Receding	34%
	Y	$\frac{1}{96} = 1\%$	Pinhead. Receding	
$\frac{786}{0}$	X	$\frac{2}{48} = 4\%$	Pinhead	Less than 10%
	Y	$\frac{4}{91} = 4\%$	Pinhead	

MOUSE	TUMOR	RESULT	SIZE OF TUMORS AND CHARACTER OF THEIR GROWTH	DIFFERENCE
$\frac{827}{0}$	V	$\frac{100}{128} = 78\%$	Majority progressively growing, hickory nut size	62%
	W	$\frac{60}{109} = 55\%$	A few reached hazelnut size. Majority receded	
	X	$\frac{15}{94} = 16\%$	Pinhead	
$\frac{770}{0}$	X	$\frac{7}{86} = 8\%$	Hazelnut to hickory nut size, progressing or receding	Less than 10%
	Y	$\frac{9}{112} = 8\%$	Pea to hickory nut, progressing or receding	
$\frac{828}{0}$	X	$\frac{18}{100} = 18\%$	Majority pinhead. Remainder pea size	Less than 10%
	Y	$\frac{9}{102} = 9\%$	Pinhead	
$\frac{841}{0}$	T	$\frac{19}{83} = 23\%$	Pinhead	23%
	W	$\frac{0}{76} = 0\%$		
	X	$\frac{26}{112} = 23\%$	Majority pinhead	
$\frac{875}{0}$	W	$\frac{14}{90} = 15\%$	Majority pinhead	Less than 10%
	X	$\frac{17}{71} = 24\%$	Majority pinhead	
$\frac{847}{0}$	X	$\frac{9}{139} = 6\%$	Pinhead	Less than 10%
	Y	$\frac{0}{123} = 0\%$		
$\frac{788}{0}$	X	$\frac{0}{94} = 0\%$		Less than 10%
	Y	$\frac{1}{77} = 1\%$	Pinhead	

MOUSE	TUMOR	RESULT	SIZE OF TUMORS AND CHARACTER OF THEIR GROWTH	DIFFERENCE
$\frac{856}{0}$	V	$\frac{0}{115} = 0\%$		Less than 10%
	X	$\frac{4}{92} = 4\%$	Pinhead	
	Y	$\frac{0}{110} = 0\%$		
$\frac{767}{0}$	T	$\frac{63}{128} = 49\%$	Pinhead to pea. Many receding	18%
	V	$\frac{35}{113} = 31\%$	Pinhead to pea. Many receding	
	Y	$\frac{75}{147} = 51\%$	Majority pea to hickory nut, and progressively growing	
$\frac{902}{0}$	W	$\frac{0}{70} = 0\%$		Less than 10%
	X	$\frac{0}{66} = 0\%$		
$\frac{803}{0}$	W	$\frac{1}{88} = 1\%$	Pinhead	10%
	Y	$\frac{11}{96} = 11\%$	Pinhead to pea. Majority receding	
$\frac{838}{0}$	V	$\frac{1}{107} = \frac{9}{10}\%$	Pinhead	Less than 10%
	X	$\frac{2}{97} = 2\%$	Pinhead	
$\frac{944}{0}$	X	$\frac{16}{123} = 13\%$	Pinhead to pea, all receding but 1	18%
	Y	$\frac{39}{126} = 31\%$	Pinhead, all receding but 3	

MOUSE	TUMOR	RESULT	SIZE OF TUMORS AND CHARACTER OF THEIR GROWTH	DIFFERENCE
$\frac{1069}{0}$	V	$\frac{9}{123} = 7\%$	Pinhead to small pea. Receding	Less than 10%
	W	$\frac{0}{113} = 0\%$		
	X	$\frac{5}{114} = \frac{9}{10}\%$	Pea to hickory nut. Progressive	
$\frac{925}{0}$	W	$\frac{0}{97} = 0\%$		Less than 10%
	X	$\frac{0}{96} = 0\%$		
	Y	$\frac{0}{83} = 0\%$		
$\frac{1241}{0}$	X	$\frac{1}{96} = 1\%$	Pea size	10%
	Y	$\frac{10}{93} = 11\%$	Pea to hickory nut	
$\frac{1269}{0}$	W	$\frac{0}{70} = 0\%$		Less than 10%
	X	$\frac{7}{103} = 7\%$	Pea to walnut. Progressive	

It is evident from the table that spontaneous growths from the same mouse give in many cases about the same percentage of daughter tumors after transplantation, though in some instances there is considerable difference in the result. It is impossible to say, however, just where the line shall be drawn between a similar and a dissimilar outcome, for it is difficult to decide what margin to allow for unavoidable experimental error. To those familiar with the enormous differences produced in biological experiments by factors that are not under the control of the investigator, 10 per cent will seem a low point at which to place the dividing line. Yet even when spontaneous tumors are

regarded as having given a similar inoculation percentage if the difference between the highest and the lowest outcome be not greater than 10 per cent, it appears that the growths of more than half (27) of the 44 mice behaved similarly after transplantation. If the more reasonable difference of 25 per cent be chosen as the point at which to divide similarly from dissimilarly growing tumors, it is found that the spontaneous neoplasms of 38 out of 44 mice resembled one another in their power (or lack of power) to grow after transplantation; in other words, multiple tumors from 38 mice out of 44 exhibited a difference of but 25 per cent or less in the number of daughter tumors. Finally, if only those tumors be regarded as similar which show a difference between their inoculation results of 50 per cent or less, the growths of 42 mice out of 44 fall in this class—for $\frac{474}{0}$ approaches so closely to 50 per cent that it may be eliminated with $\frac{827}{0}$.

Thus, when all inoculation results below 25 per cent are regarded as equal, spontaneous neoplasms from approximately nine-tenths of the mice are found to yield the same outcome after transplantation; this suggests that adaptation is an important factor in the establishment of daughter tumors, and the hypothesis is strengthened by the fact that the growths from more than half the mice still show a similar number of daughter tumors when only those percentages are declared equal which vary by 10 per cent or less.

Two mice were found, however, whose tumors gave inoculation results differing by as much as 49 and 62 per cent respectively, which indicates that spontaneous neoplasms may exhibit variations in their growth energy after transplantation. Such a supposition is borne out by mouse $\frac{827}{0}$, where three grades of proliferative energy appear to have been present in her three growths, shown not only by the inoculation percentage but also by the manner in which the daughter tumors grew. Thus X produced 16 per cent of receding daughter tumors, none larger than a pinhead; W gave rise to 55 per cent of pea-size growths, of which a few reached the dimensions of a hazelnut though the majority receded; and V afforded 78 per cent of tumors, of which the majority grew progressively and had reached the size of a hickory nut at the end of eight weeks.

Such variations, however, do not appear to be the rule, for in only 6 mice out of 44 did the inoculation percentages of multiple neoplasms vary by more than 25 per cent.

Hence it is probable that the factor which usually decides whether or not a spontaneous tumor can be successfully transplanted, is adaptation. But it is equally probable that multiple spontaneous neoplasms occasionally vary in their proliferative energy; in most of them this is so low after transplantation as to be masked by a failure on the part of the tumor cells to adapt themselves, though in the case of a few growths it rises so high as to transcend the adaptive power of these elements.

It may be pointed out, in passing, that the recurrences in mouse $\frac{158}{0}$ furnished approximately the same proportion of daughter tumors as the primary growths, and that no parallel could be observed between the proliferative rate of spontaneous neoplasms in the mouse affected, and the percentage of daughter tumors. The more slowly growing tumor sometimes produced the greater percentage.

SUMMARY

The presence (or absence) of power to adapt themselves to new hosts appears to be a deciding factor in the success (or failure) of most spontaneous mouse carcinomata after transplantation. In a few such neoplasms, however, a high proliferative energy appears to aid in gaining a foothold.

REFERENCES

(1) Ehrlich, P.: Arb. a. d. königl. Inst. für exp. Therap. zu Frankfurt a. M., 1906, Heft l, 90.
Ztschr. f. Krebsforsch., 1907, v, 62.

(2) Apolant, H.: Ztschr. f. allg. Physiol., 1909, ix, Sammelreferat, 69.

(3) Bashford, E. F., Murray, J. A., Haaland, M., and Bowen, W. H.: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, pp. 109, 161, 270.

(4) Murray, J. A.: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, pp. 109, 161.

(5) Bashford, E. F., Murray, J. A., and Cramer, W.: Proc. Roy. Soc., Series B, 1907, lxxix, 164.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, pp. 326, 337.

(6) Haaland, M.: Berl. klin. Wchnschr., 1907, xliv, 713.
(7) Fleisher, M. S., and Loeb, L.: Jour. Med. Research, 1916, n. s. xxix, 1.
(8) Murray, J. A.: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, p. 106.
(9) Russell, B. R. G.: Jour. Path. and Bacteriol., 1910, xiv, 347.
(10) Bashford, E. F.: Fourth Sci. Report, Imperial Cancer Research Fund, London, 1911, p. 131.
(11) Gierke, E.: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, p. 118.
(12) Haaland, M.: Fourth Sci. Report, Imperial Cancer Research Fund, London, 1911, p. 25.
(13) Murray, J. A.: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, p. 98.

A MAMMARY CARCINOMA IN THE CAT

SHIGEMITSU ITAMI

From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director

Received for publication, January 2, 1918

Mammary carcinomata of the cat are not of particularly rare occurrence, for a number of cases are to be found in the literature of the past twenty-seven years. One of these was reported by Spencer (1), five by Sticker (2), one by Petit (3), one by Leyden (4), one by Petit and Cornil (5), four by Murray (6), one by Petit and Finzi (7), and one by Clunet (8). To these may be added a few cases cited by Wolff (9), and by Petit (10), whose original articles, however, are not available to the writer.

The example to be described in this paper is that of a large female cat received through the kindness of Dr. Benjamin White of Otisville, N. Y., on March 23, 1917.

In the right axillary mamma there was a hard, nodular, tumor about 10 by 3 by 2 cm., containing a few small cysts, and adherent to the skin which, toward the lower pole of the growth, presented two ulcers involving the lowermost axillary nipple. These resembled cancerous ulcers in the human subject, their edges being raised and scalloped and the floor formed of firm granulation tissue discharging a foul ichor. Toward the upper pole, the tumor grew narrow, passing by the upper nipple and extending into the right axilla where it broke up into a string of separate nodules each about the size of a green pea.

At autopsy, on March 26, 1917, it was found that the growth was not adherent to the underlying musculature. The axillary lymph-nodes, as well as the supracondylar and one in the neighboring connective tissue, were enlarged. The lungs contained a few shot-sized nodules, these being more prevalent in the middle lobe of the right lung, which appeared to be carnified and con-

tained about six such lesions. Except for one or two small shot-like nodules in the liver, which might have been metastases, the rest of the body was free from secondary growths. There was one fetus in the right horn of the uterus.

Sections removed from the tumor appear upon microscopic examination to be composed of papillary cysts with a stroma of thickened connective tissue. The cysts are almost completely filled by a considerable number of elongated processes (fig. 1)

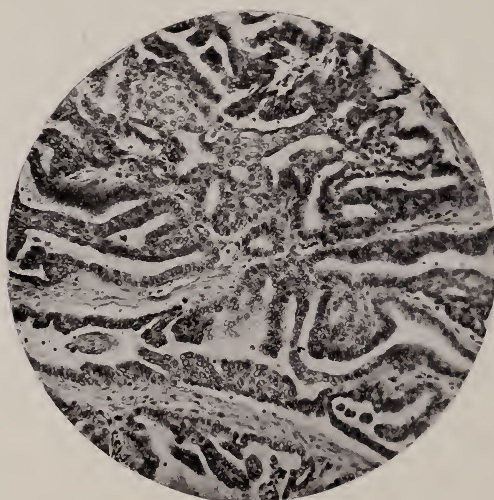


Fig. 1. Showing Papillary Nature of the Tumor

springing from their walls, which branch out irregularly and appear to anastomose with one another. The walls of the cysts are made up of well-defined connective tissue lined with epithelium, the elements of which consist of large cells ranging in shape from cuboidal to cylindrical, usually arranged in one layer, and containing large oval nuclei; in some places, however, especially at the bases of the papillae, the epithelial lining is exceedingly thick. Mitotic figures are prominent.

While papillary outgrowth is so luxuriant, invasion of the surrounding tissue (fig. 2) is also a noteworthy feature, the subjacent lymph-channels, or perhaps the connective tissues underlying the bases of the papillae, being frequently attacked.

The stroma of the tumor is abundant and firm, forming a prominent part of the growth; in some portions it is so dense as to suggest scar tissue. The fibers are arranged in irregular bands, and it is from those forming the walls of the cysts that fibrils to the papillae branch out. There are many areas of circumscribed round cell infiltration throughout the stroma. The tumor has no capsule and fades out indefinitely into the surrounding tissues.

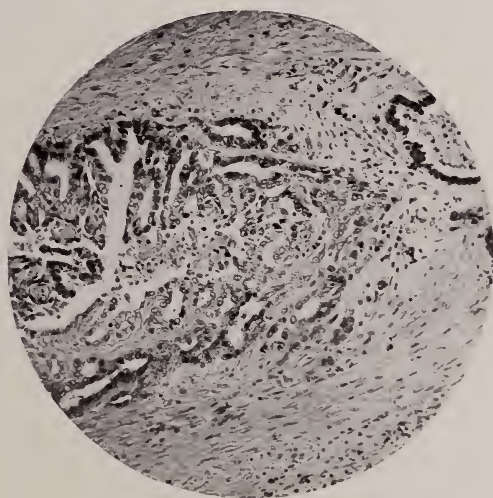


Fig. 2. Invasion of the Surrounding Tissues

In the supracondylar nodule above referred to, the lymphoid tissue of the lymph-node is displaced by tumor.

The pulmonary metastases contained few cysts with intracystic projections and well-defined walls, but the dominant feature here is that of a papillary carcinoma without cyst formation. The papillae grow into and fill the air spaces of the lung, without actual involvement of their wall, and except for a moderate dilation of the blood-vessels and a slight round-cell infiltration, the interlobular tissue around the nodules remains unaffected.

The nodules in the liver prove to be fatty infiltration.

The corresponding mamma on the opposite side, which did not show any macroscopic changes at autopsy, was examined microscopically to see whether changes like those described by Haaland (11) in the mouse could be discovered. The coexisting pregnancy naturally obscured somewhat the conditions present in the organ, but there could be no doubt that it was the seat of an extensive papillary cystadenomatous change, or that certain regions were carcinomatous; whether these areas represented new tumors, however, or metastases from that in the other breast, could not be decided.

REFERENCES

(1) Spencer, W. G.: Tr. Path. Soc., London, 1890, xli, 400.

(2) Sticker, A.: Ueber den Krebs der Tiere (Sonder Abdruck aus dem Arch. f. klin. Chir., Bd. 65), Berlin, 1902, pp. 79 and 126.

(3) Petit, G.: Bull. Soc. anat. de Paris, 1902, iv, 409.

(4) Leyden, E. von: Ztschr. f. klin. Med., 1904, lii, 409.

(5) Petit, G. and Cornil, V.: Bull. Soc. anat. de Paris, 1906, lxxxi, 208.

(6) Murray, J. A.: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, p. 44.

(7) Petit, G. and Finzi, G.: Bull. de l'Assoc. franç. pour l'étude du cancer, Paris, 1910, vii, 305.

(8) Clunet, J.: Recherches expérimentales sur les Tumeurs malignes, Paris, 1910, p. 4.

(9). Wolff, J.: Die Lehre von der Krebskrankheit, Jena, 1913, Teil iii, 1ste Abt., 253.

(10) Petit, G.: Bull. Soc. centr. de Méd. vét., 1914–15.

(11) Haaland, M.: Fourth Sci. Report, Imperial Cancer Research Fund, London, 1911, 30–37.

AN INVESTIGATION OF THE POWER OF MESODERMAL DERIVATIVES TO IMMUNIZE MICE AGAINST TRANSPLANTABLE TUMORS

SHIGEMITSU ITAMI

From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director

Received for publication, January 21, 1918

When the various tissues which have been used to immunize mice against transplantable tumors are passed in review, it is found that representatives of all three germ layers have the power to confer resistance, with the exception of one ectodermal derivative, i.e., the crystalline lens. While both Schöne (1) and Borrel and Bridré (2) have asserted that the injection of testis will not bring about the refractory state, a more recent investigator (3) believes that the testis will confer resistance; furthermore, an experiment conducted in this laboratory by Drs. Bullock and Rohdenburg, and hitherto unpublished, shows that the testis is not without immunizing power, at least in the rat. It has, therefore, been entered in the table (table 3) accordingly (+).

The failure of mouse lens to produce immunity in the experiment of Uhlenhuth and Weidanz (4) is an interesting phenomenon in view of the constancy with which most other tissues call forth the refractory state. Its inactivity cannot be ascribed to an insufficient amount, at any rate, for three doses (1 cc. each) of an emulsion were given; nor was tumor inoculation undertaken at an unfavorable time, for it followed twenty-eight days after the first treatment with lens, and eight days after the last, a period within which immunity should still be high, as Woglom (5) has shown.

The experiment was accordingly repeated by the present writer in another species of animal, rats treated with 0.05 cc.

of an emulsion of rat lens, being inoculated one, two, three, or four weeks afterwards with 0.003 gram[1] of the Flexner-Jobling rat carcinoma by the needle method. The outcome was identical with that recorded by Uhlenhuth and Weidanz, for not the slightest indication of immunity could be discovered in any of the experiments, of which figure 1 reproduces a perfect sample.

FIG. 1. EXPERIMENT $\frac{FRC}{19C}$

No immunity to carcinoma three weeks after treatment with 0.05 cc. of an emulsion of rat lens. Tumor dose, 0.003 gram by the needle method.

It may be objected that the amount of lens administered was too small. But the experience of Bullock and Rohdenburg (6) shows that this quantity of rat embryo skin emulsion will produce a distinct resistance against the same dose (0.003 gram) of the same tumor (Flexner-Jobling rat carcinoma). Still it is possible that the amount of lens, while not actually too small, was relatively insufficient; for the lens contains but a few cells, and the authors (6) just cited have demonstrated that tissues like cartilage and bone, which have a minimum of cellular material

[1] In previous publications from the Imperial Cancer Research Fund and from this laboratory, the inoculation dose, when the needle method was used, has been estimated as 0.01 or 0.02 gram; but such grafts have recently been found, as a matter of fact, to weigh about 0.002 and 0.003 gram respectively.

and a maximum of matrix, do not produce a high resistance. It may be, therefore, that the lens fails for a similar reason, and the comparative failure of all three may perhaps prove to be another vindication of Haaland's assertion (7) that growth of the cells after their introduction is an essential for the production of immunity.

It will be appreciated that the preparation of an emulsion of rat lens in sufficient amount is an expensive and laborious process, and that for this reason a large quantity could not be obtained at the present time owing to war conditions; it may be possible at some future time, however, to repeat one of these experiments, using a larger dose.

The brain will perhaps have to be included among the tissues incapable of eliciting distinct resistance; for, while Borrel and Bridré (8) are said to have succeeded in immunizing with brain, a repetition of the experiment at this laboratory has given entirely negative results. Since the French observers conducted their investigation some time ago, when the importance of dosage and time interval was hardly yet recognized, these more recent experiments, in which both have been taken into account, afford a result that is, perhaps, nearer the truth. Accordingly, the brain has been entered in table 3 as possibly negative, a definite decision, as in the case of the lens, being reserved for the future.

The following experiments were undertaken to determine whether any other tissues share with the lens, brain, cartilage, and bone, their inability to elicit a vigorous immunity. Two mesodermal derivatives—muscle and lymph-node—were chosen. In order that the findings might not be vitiated by the presence of blood in these tissues, the greatest care was taken not to injure large vessels during the removal of the material; the small amount of blood contained in the muscle and the lymph-node themselves may be regarded as insufficient to produce any immunity.

Muscles or lymph-nodes were removed from healthy mice, emulsified, and administered subcutaneously in the left axilla of normal young adult mice in doses of 0.05 cc. (lymph-node) and 0.1 cc. (muscle). Into the opposite axillae of the animals

thus injected, and into untreated normal controls of the same size and breed which had been kept aside under identical conditions, grafts (0.003 gram) of tumor were implanted one to four weeks after the preliminary treatment. The three tumors employed, 63, 11 and 48, are transplantable mammary adenocarcinomata.

Daughter tumors were charted first ten days after the inoculation of the grafts, and subsequently at intervals of one week, and the results thus obtained are reproduced in a number of charts and a table (table 1). These, however, represent but a few of the experiments; it is unnecessary to publish the whole series because the results were similar in all cases.

It will be seen that preliminary treatment with lymph-node calls forth a distinct resistance (figs. 2 to 5) to two carcinomata, though the immunity is perhaps of shorter duration than that induced by some other tissues. The vigorous refractory state obtained with lymph-node, however, obviously has no bearing upon questions regarding the rôle of the lymphocyte in immunity, for an equally high protection can be brought about with other materials.

Muscle, on the other hand, does not induce resistance in the amount employed (figs. 6 to 8). This material, therefore, appears to share with bone, cartilage and the crystalline lens their inability to evoke an efficient immunity against cancer. Why this should be so is not clear; certainly the proportion of cells in muscle is greater than in bone, cartilage, or lens. It may be that the cells of muscle are not readily broken down and absorbed after introduction into the subcutaneous tissues.

The fact having been established that muscle fails to elicit immunity to carcinoma, it becomes necessary to know whether it will confer resistance against a growth which is, like itself, of mesodermal origin, or whether it will resemble such an efficient immunizer against carcinoma as epithelium is known to be, in being unable to produce resistance against the majority of sarcomata. Mice that had been treated with muscle were therefore tested with two sarcomata, the Ehrlich and the Crocker Fund No. 180, both known to be insusceptible to 0.1 cc. of embryo

TABLE 1

CARCINOMA	INTERVAL	MICE	TREATED WITH					
			Muscle			Lymph-node		
			Survived	Negative		Survived	Negative	
				Number	Per cent		Number	Per cent
63	1 week	Treated	22	5	22.72	23	21	91.30
		Controls	22	1	4.58	22	1	4.58
	2 weeks	Treated	22	7	31.81	22	18	81.81
		Controls	21	1	4.76	21	4	19.04
	3 weeks	Treated	18	5	27.77	23	7	30.43
		Controls	24	2	8.33	23	0	0.00
	4 weeks	Treated	24	9	37.50	16	3	18.75
		Controls	24	4	16.66	24	2	8.33
11	1 week	Treated	23	6	26.08	10	10	100.00
		Controls	24	8	33.33	10	5	50.00
	2 weeks	Treated	18	6	33.33	20	20	100.00
		Controls	24	6	25.00	22	9	40.00
	3 weeks	Treated	20	7	35.00	9	8	88.88
		Controls	17	8	47.05	12	4	33.33
	4 weeks	Treated	23	7	30.43	22	18	81.81
		Controls	23	10	43.47	24	8	33.33
48	1 week	Treated	23	11	47.82	23	19	82.60
		Controls	22	6	27.27	23	1	4.34
	2 weeks	Treated	21	18	38.09	22	18	81.81
		Controls	23	0	0.00	10	4	20.00
	3 weeks	Treated	19	6	31.59	18	15	83.33
		Controls	21	7	33.33	17	7	41.17
	4 weeks	Treated				19	13	68.42
		Controls				21	3	14.28

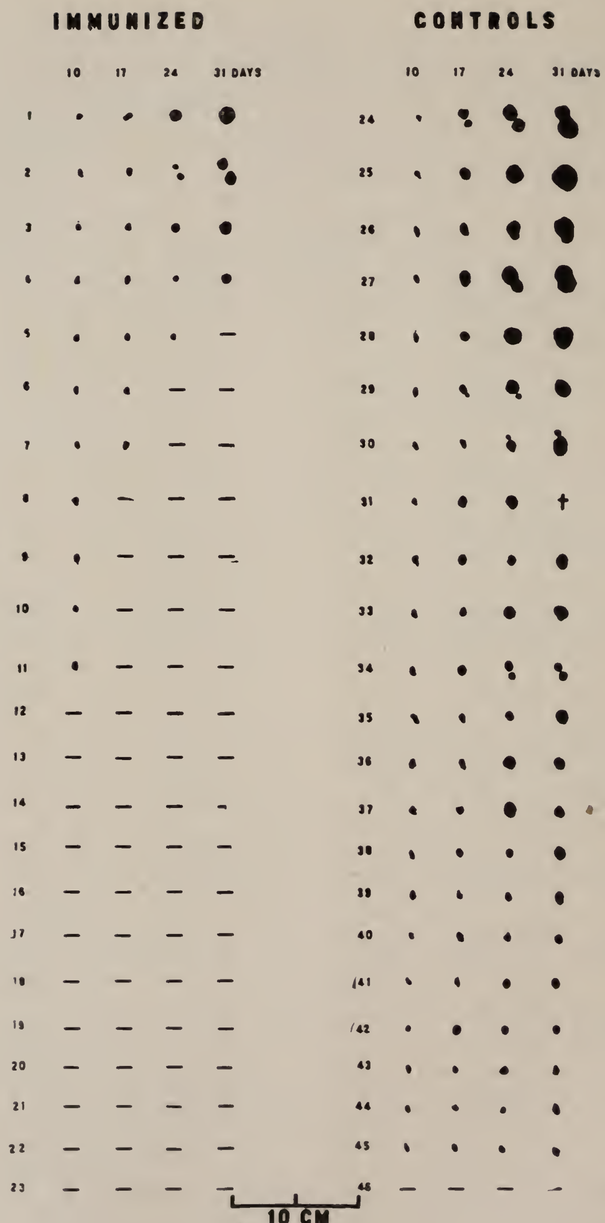


FIG. 2. EXPERIMENT $\frac{48}{33B}$

Distinct immunity to carcinoma one week after treatment with 0.05 cc. of an emulsion of mouse lymph-node. Tumor dose, 0.003 gram by the needle method.

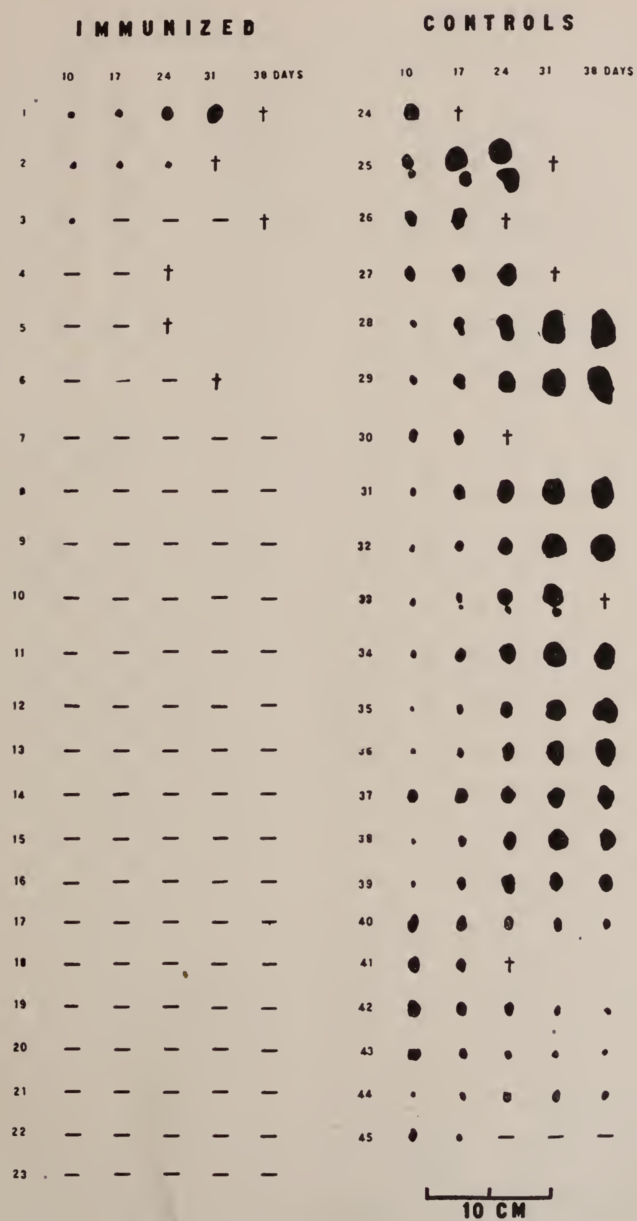


FIG. 3. EXPERIMENT $\frac{63}{136L}$

Distinct immunity to carcinoma one week after treatment with 0.05 cc. of an emulsion of mouse lymph-node. Tumor dose, 0.003 gram by the needle method.

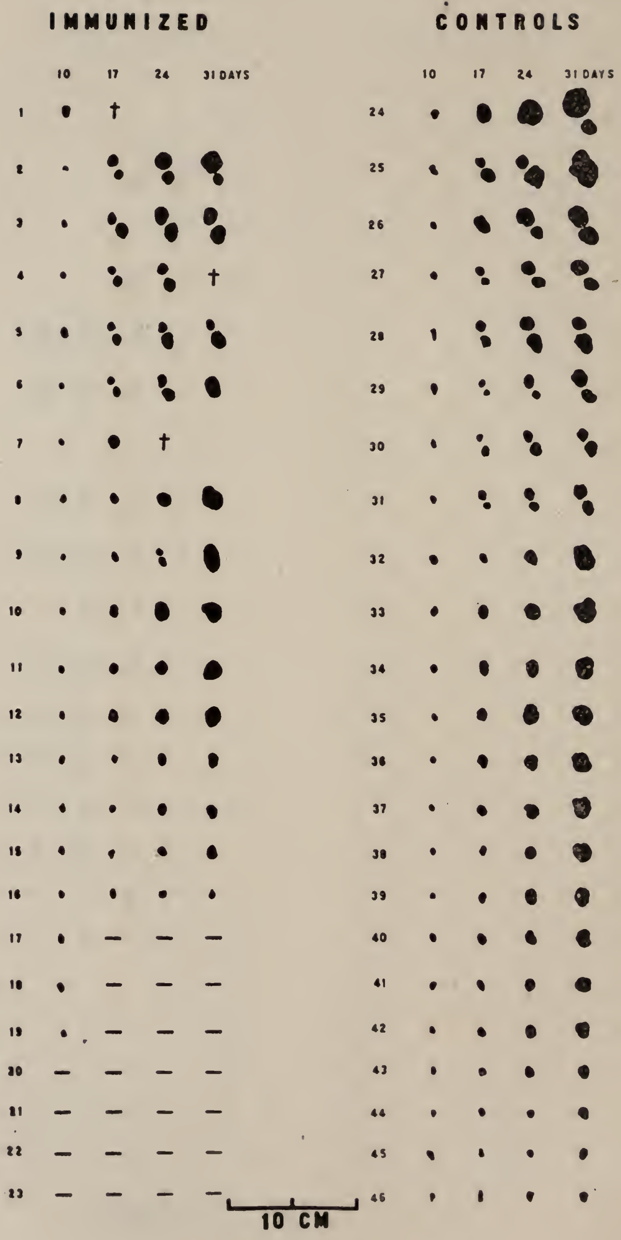


FIG. 4. EXPERIMENT $\frac{63}{141H}$

Slight immunity to carcinoma three weeks after treatment with 0.05 cc. of an emulsion of mouse lymph-node. Tumor dose, 0.003 gram by the needle method.

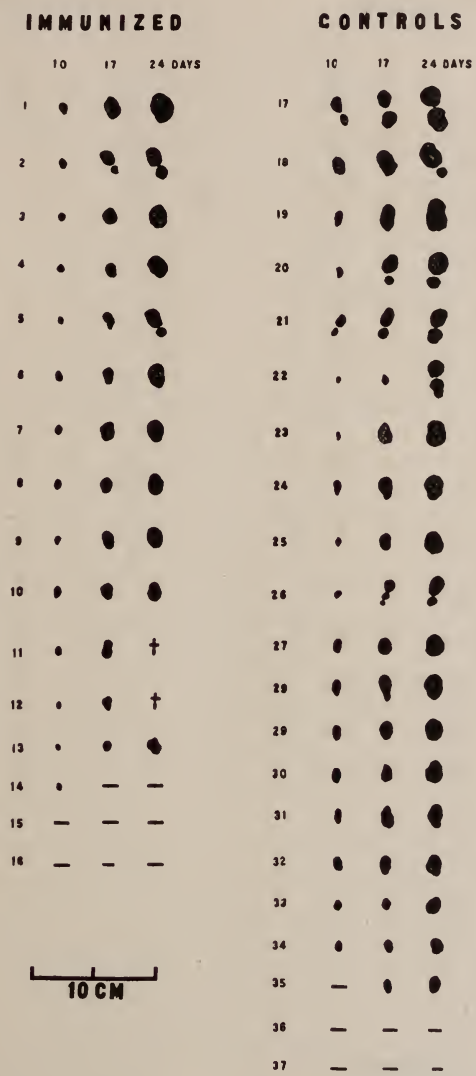


FIG. 5. EXPERIMENT $\frac{63}{138Q}$

Practically no immunity to carcinoma four weeks after treatment with 0.05 cc. of an emulsion of mouse lymph-node. Tumor dose, 0.003 gram by the needle method.

FIG. 6. EXPERIMENT $\frac{11}{44E}$

No immunity to carcinoma two weeks after treatment with 0.1 cc. of an emulsion of mouse muscle. Tumor dose, 0.003 gram by the needle method.

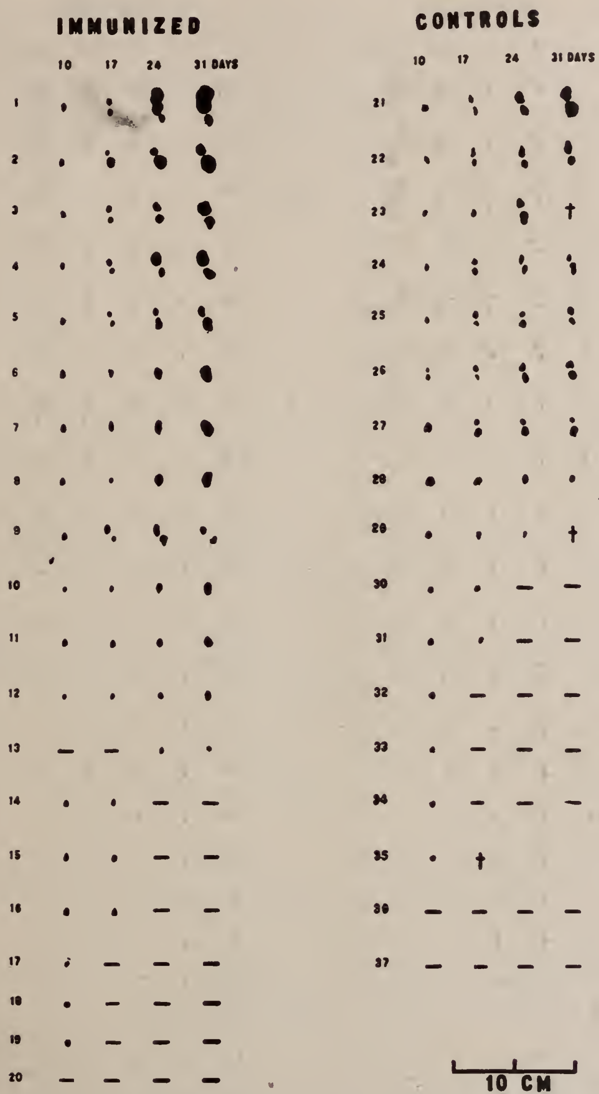


Fig. 7. Experiment $\frac{11}{44B}$

No immunity to carcinoma three weeks after treatment with 0.1 cc. of an emulsion of mouse muscle. Tumor dose, 0.003 gram by the needle method.

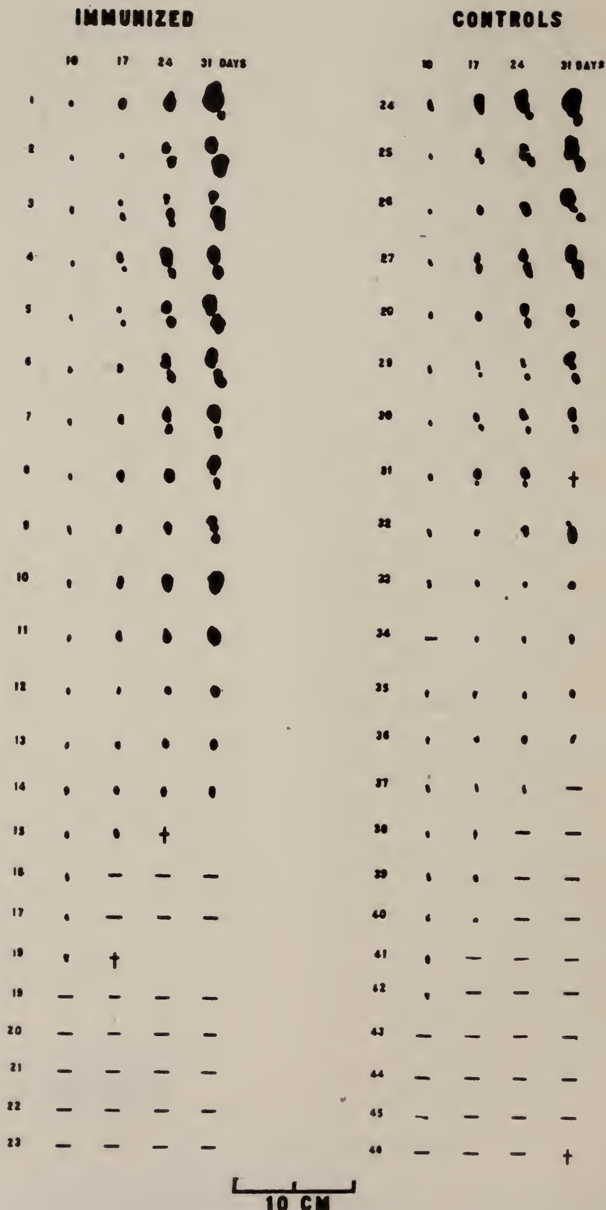


FIG. 8. EXPERIMENT $\frac{11}{44D}$

No immunity to carcinoma four weeks after treatment with 0.1 cc. of an emulsion of mouse muscle. Tumor dose, 0.003 gram by the needle method.

skin (figs. 10 and 12, and "Treated controls" in table 2). As figures 9 to 13 and table 2 show, muscle is impotent to protect against sarcoma, and the same is true of lymph-node. In other words, the mesodermal derivatives chosen do not protect against two neoplasms of mesodermal origin, although the interval

TABLE 2

SARCOMA	INTERVAL	MICE	TREATED WITH: Muscle — Survived	Muscle — Negative — Number	Muscle — Negative — Per cent	Lymph-node — Survived	Lymph-node — Negative — Number	Lymph-node — Negative — Per cent
180	1 week	Treated	21	6	28.75	24	11	45.83
		Controls	23	1	4.34	21	2	9.52
	2 weeks	Treated	22	4	18.18	20	0	0.00
		Controls	24	0	0.00	21	0	0.00
		"Treated controls"				15	0	0.00
	3 weeks	Treated	14	1	7.14	17	2	11.76
		Controls	20	0	0.00	20	2	10.00
		"Treated controls"	22	8	36.36			
	4 weeks	Treated	33	2	6.06	11	0	0.00
		Controls	35	1	2.85	20	1	5.00
		"Treated controls"				14	1	7.14
E. S.	1 week	Treated	24	0	0.00	16	1	6.25
		Controls	19	0	0.00	19	0	0.00
		"Treated controls"	19	0	0.00			
	2 weeks	Treated	23	0	0.00	22	0	0.00
		Controls	22	1	4.58	22	1	4.58
		"Treated controls"	22	4	18.18			

between treatment and tumor inoculation was inside the limits of the period during which immunity to sarcoma can sometimes be elicited (9).

In table 3 all the tissues that have now been tested as to their immunizing power are arranged according to derivation. The failure of cartilage, bone, and muscle is not due to their meso-

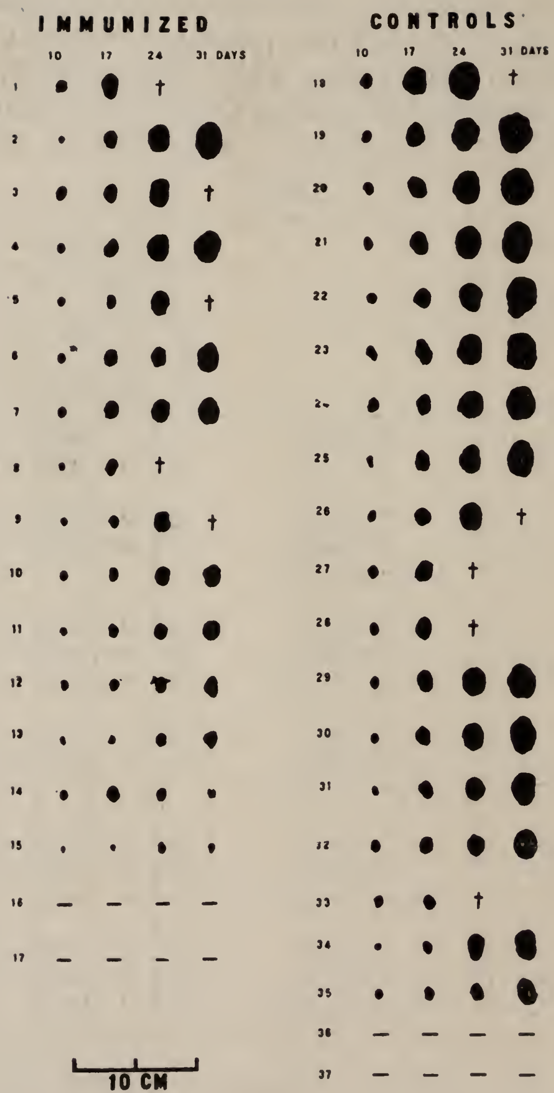


FIG. 9. EXPERIMENT $\frac{180}{26H}$

No immunity to sarcoma three weeks after treatment with 0.05 cc. of an emulsion of mouse lymph-node. Tumor dose, 0.003 gram by the needle method.

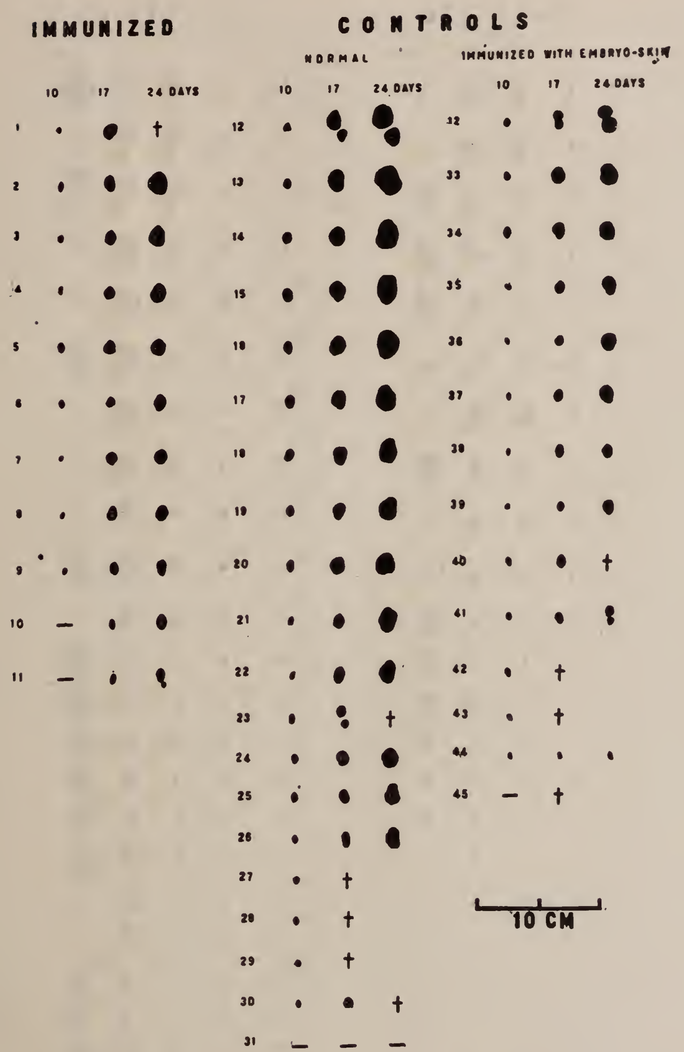


FIG. 10. EXPERIMENT $\frac{180}{431}$

No immunity to sarcoma four weeks after treatment with 0.05 cc. of an emulsion of mouse lymph-node. Tumor dose, 0.003 gram by the needle method. It will be noted that embryo skin also fails to produce resistance against this sarcoma.

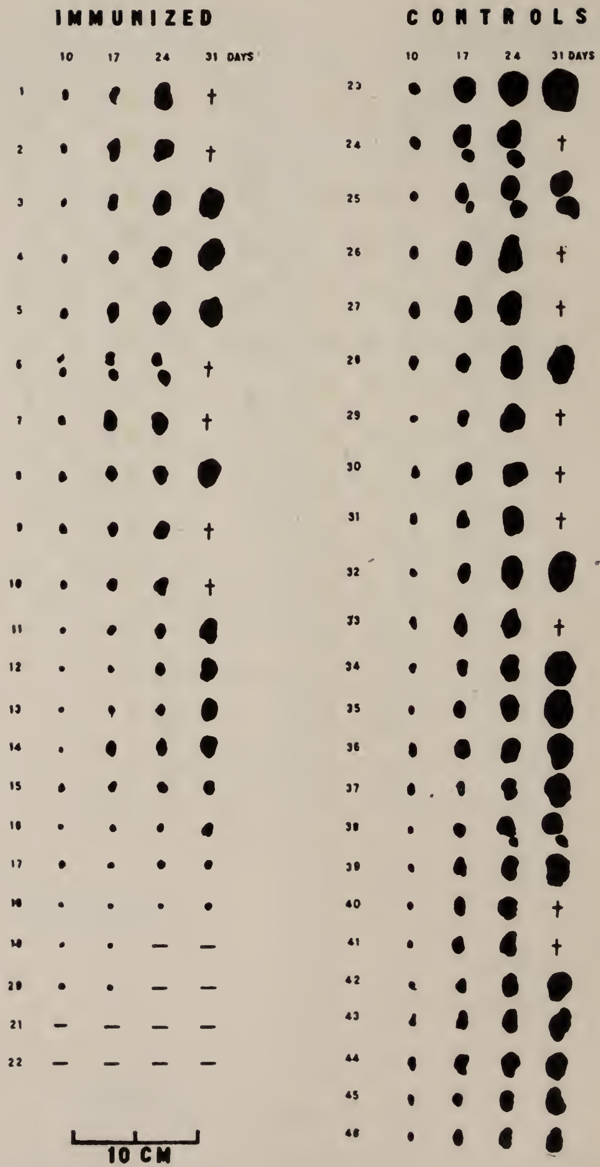


FIG. 11. EXPERIMENT $\frac{180}{26D}$

No immunity to sarcoma two weeks after treatment with 0.1 cc. of an emulsion of mouse muscle. Tumor dose, 0.003 gram by the needle method.

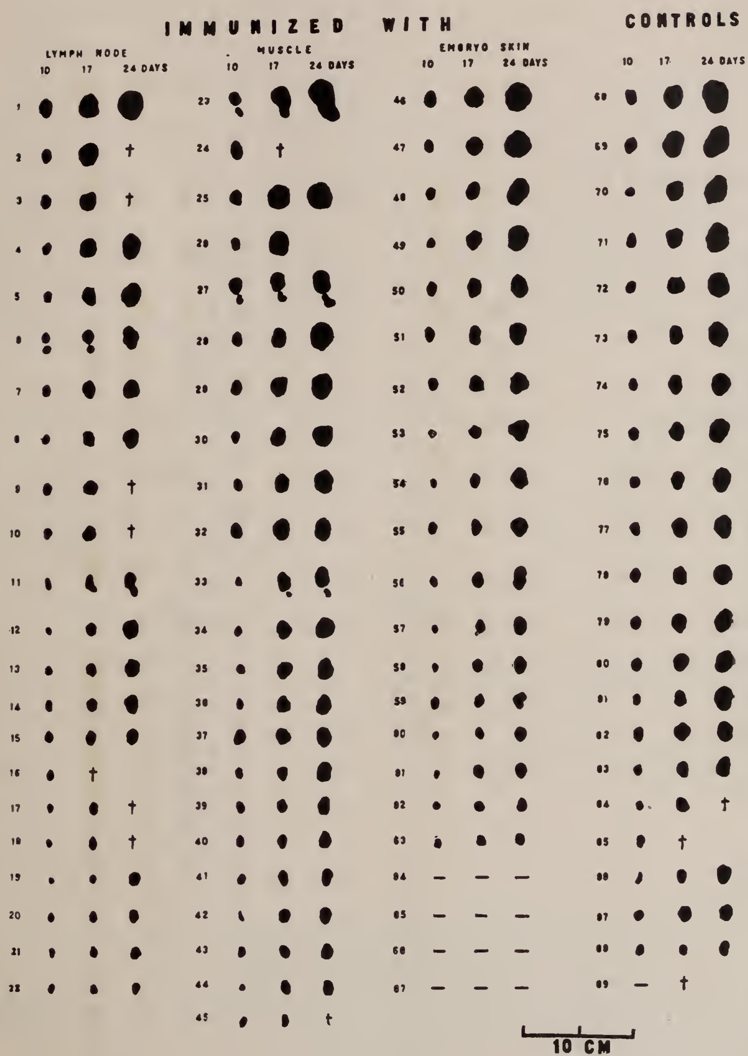


FIG. 12. EXPERIMENT $\frac{ES}{60G}$

No immunity to sarcoma two weeks after treatment with 0.1 cc. of an emulsion of mouse muscle. Tumor dose, 0.003 gram by the needle method. It will be noted that lymph-node and embryo skin also fail to produce resistance against this sarcoma.

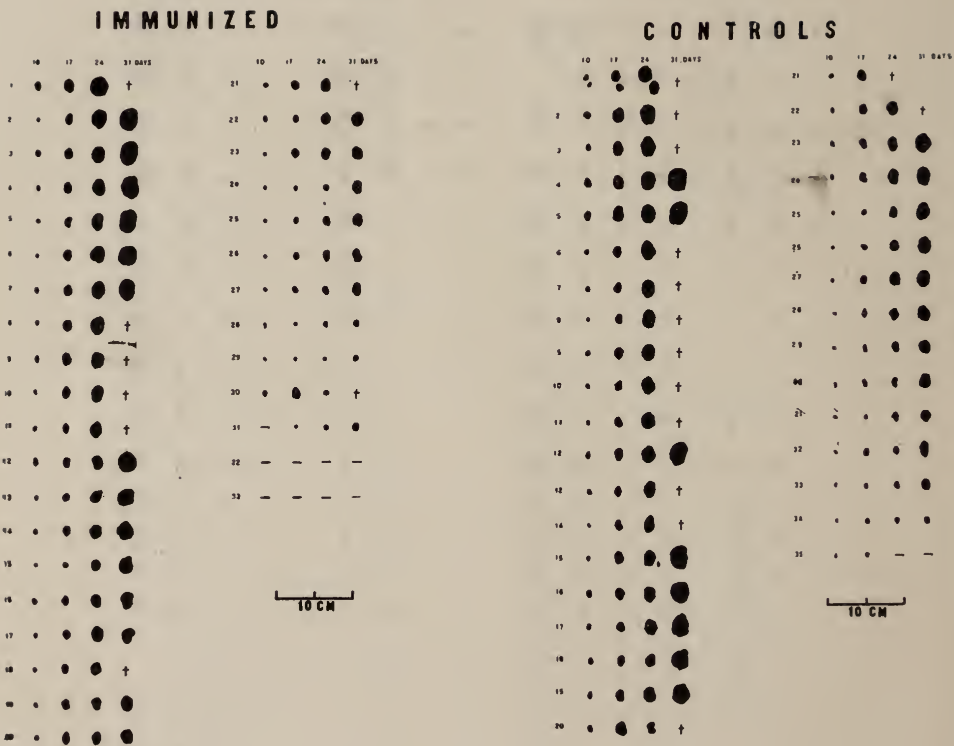


FIG. 13. EXPERIMENT $\frac{180}{26F}$

No immunity to sarcoma four weeks after treatment with 0.1 cc. of an emulsion of mouse muscle. Tumor dose, 0.003 gram by the needle method.

dermal origin, for other materials derived from the same source are fully potent; furthermore, the inability is shared by at least one tissue descended from the ectoderm. As has already been pointed out, lens, cartilage, and bone contain comparatively few cells in contrast with those tissues which are efficient in eliciting the refractory state; yet muscle, which is more cellular, also fails completely. This can hardly be referred to its high differentiation, for the skin, which is the most active immunizer known, is perhaps as highly differentiated a tissue. The need for further investigation is obvious, and this will be

TABLE 3

Tissues which will immunize against transplantable carcinoma are followed by the plus sign

Tissue	
Ectoderm	
Fetal skin	+
Mamma	+
Lens	−
Brain	−?
Mesoderm	
Spleen (containing blood)	+
Blood	+
Testis	+
Kidney	+
Cartilage	−
Bone	−
Muscle	−
Lymph-node	+
Entoderm	
Liver	+
All Layers	
Embryo	+
Extraembryonic Ectoderm and Mesoderm	
Placenta (washed free of blood)	+

undertaken as soon as conditions permit; indeed, it is even now under way. The present communication, therefore, is to be regarded as scarcely more than a preliminary report.

SUMMARY

Preliminary treatment with normal tissues containing but few cells, whether they be of ectodermal or mesodermal origin, fails to induce immunity to transplantable carcinomata. Muscle, also, though this is more cellular, is inactive, for some reason at present unknown.

Lymph-node, on the contrary, has the power to elicit a high resistance against transplantable carcinomata.

The mesodermal tissues investigated have no power to immunize against two connective tissue tumors employed, failing, like the skin, to protect against sarcoma.

REFERENCES

(1) Schöne: Münch. med. Woch., 1906, liii, 2519.

(2) Borrel and Bridré: Bull. de l'Inst. Past., 1907, v, 605. Ann. de l'Inst. Past., 1907, xxi, 769.

(3) Russell: Fifth Sci. Report, Imperial Cancer Research Fund, London, 1912, 23.

(4) Uhlenhuth and Weidanz: Arb. a. d. k. Gsndhtsamte., 1909, xxx, 443.

(5) Woglom: Jour. Exper. Med., 1912, xvi, 629.

(6) Bullock and Rohdenburg: Jour. Cancer Research, 1917, ii, 456.

(7) Haaland: Proc. Roy. Soc., Series B, 1909–10, lxxxii, 293; Lancet, 1910, i, 787.

(8) Borrel and Bridré: Cited by Bashford, Murray, and Haaland, Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 376.

(9) Tsurumi: Jour. Path. and Bacteriol., 1915–16, xx, 76.

PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH

ELEVENTH ANNUAL MEETING

Held in Philadelphia, March 28, 1918

1. Report of the Council

The following members were present at this meeting: Dr. F. C. Wood, president; Dr. William H. Woglom, secretary and treasurer; Dr. James Ewing, Dr. H. Gideon Wells. Absent, Dr. Harvey R. Gaylord, and Dr. James B. Murphy.

The report of the treasurer, showing a balance on hand of $275.19, was read and accepted.

Dr. E. R. Le Count, of Rush Medical College, Chicago, was elected as councillor to complete the term of the late Dr. Richard Weil, whose period in office would have expired in 1921.

Dr. Robert B. Greenough, of Boston, was elected to the Council to succeed Dr. James Ewing, automatically retired by the time limit.

The following officers were elected for the ensuing year: Dr. E. R. Le Count, president; Dr. H. Gideon Wells, vice-president; Dr. William H. Woglom, secretary and treasurer (re-elected).

The present members of the Council, with the years of their retirement, are:

Dr. Harvey R. Gaylord, 1919	Dr. F. C. Wood, 1922
Dr. H. Gideon Wells, 1920	Dr. James B. Murphy, 1923
Dr. E. R. Le Count, 1921	Dr. William H. Woglom, 1924

Dr. Robert B. Greenough, 1925

Dr. William H. Woglom was elected managing editor to succeed the late Dr. Richard Weil, and Dr. Frederick Prime was appointed associate editor. Dr. James Ewing was placed upon the Editorial Board, of which the other members were retained in office for the ensuing year. This board is now composed, therefore, as follows:

Dr. William H. Woglom, Columbia University	Dr. Leo Loeb, Washington University
Dr. Frederick Prime, Columbia University	Dr. Ernest E. Tyzzer, Harvard University
Dr. Joseph C. Bloodgood, Johns Hopkins University	Dr. H. Gideon Wells, University of Chicago

Dr. James Ewing
Cornell University

NEW MEMBERS

Honorary Member

Dr. Katsusaburo Yamagiwa, Imperial University, Tokyo, Japan

Active Members

Dr. Kenneth M. Lynch, Charleston, South Carolina
Dr. Angelo Roffo, Buenos Aires, Argentine Republic
Dr. Douglas Symmers, New York

Dr. F. W. Baeslack, Detroit, Mich., was advanced from associate to active membership.

The resignations of the following members were accepted: Dr. E. V. L. Brown, Dr. L. D. Bristol, Dr. G. N. Calkins, Dr. P. Rous, Dr. H. K. Oliver, Dr. P. Woolley, Dr. J. Bowen, Dr. H. Smith.

The secretary reported that he did not carry out the instructions of last year regarding the preparation of a printed list of subscribers to be sent to all members, with a request that they endeavor to secure new members, because on the day after the meeting the United States declared that a state of war existed with Germany. He therefore regarded it as unwise to incur the expenses incidental to a subscription campaign. In this he was sustained by the Council.

Dr. Wells reported that progress has been made in arranging a federation of our society with the American Association of Pathologists and Bacteriologists and the American Association of Immunologists.

In opening the morning session, Dr. Wood referred to the blow sustained by the Association in the loss of Dr. Richard Weil, who had been interested in the early progress of the society and had for some years served as Editor of the Journal. He had regularly attended the meetings, had read papers and taken part in the discussions, and had been at one time President. He did what all would like to do—volunteered in the service of his country; and, while working at one of the camps in the South on the pneumonia question, was himself stricken with the disease and died after a brief illness.

It was moved and seconded that the Chair designate a committee to inscribe a memorial for transmission to Dr. Weil's family. The president accordingly appointed Dr. H. G. Wells and Dr. James Ewing.

2. The Lymph-Nodes in Immune Rats

Dr. William H. Woglom (New York):

SUMMARY

When lymph-nodes from rats with progressively growing or receding tumors are compared with those from normal rats, it is found that except for some slight hyperplasia of the germinal centers in the lymph-nodes from the tumor-bearing animals there is no morphological differ-

ence. Whether this change is due to the presence of the tumor or not, it is impossible to say at present, nor can its significance be forecast. The investigation is in progress.

3. Some Late and Distant Effects of Radium

Dr. James Ewing (New York):

SUMMARY

Ever since Dominici employed filtration of the soft rays of radium, this method has been universally used to secure the deeper curative effects of radium in the treatment of cancer. The method is generally very effective in its proper field, but there are certain limitations in the use of heavily filtered radium which it is important to recognize.

During the past few years I have encountered in operative and post-mortem material at the Memorial Hospital in New York, evidences of the late and distant effects of large doses of heavily filtered radium which are of importance in controlling the use of this method. That the application of a large amount, 500 mgm. to 1 gram of radium, filtered through lead and applied for twelve to twenty-four hours, at a distance of 5 to 10 cm. from the skin, is capable of causing definite regressive changes in deep seated tumors I have repeatedly observed. Among the tumors most susceptible to this action may be mentioned mediastinal lympho-sarcoma, carcinoma of the lung, and abdominal metastases of carcinoma of the testis. In the latter type of growth some very remarkable and gratifying results not infrequently follow the use of radium, but I have observed certain definite conditions caused by this tumor which can not be successfully met by radium. Thus in one very bulky metastasis, the tumor broke down rapidly; the growth was the seat of an extensive hemorrhage and the patient died, largely as a result of the loss of blood. A less vigorous application with slower effect might have resulted more favorably. In two other cases similar treatment was given without any effect upon the size of the tumor, and autopsies disclosed that these growths were of large size, firm and fibrous, encasing the great vessels and causing edema of the limbs. With this anatomical condition to deal with, it is quite impossible to expect radium to cause any great reduction in the size of a neoplasm. The most it could do would be to cause fibrosis and cicatrization which, under the circumstances, would only aggravate the constriction of the vessels. In a great many conditions a similar deposit of fibrous connective tissue prevents any marked visible effect of radium. In fact it may be stated, as a rule, that if diminution in size is to be taken as the indication of therapeutic success, this rule can be applied only to cellular, not to fibrous tumors.

In the case of osteogenic sarcoma, where there is often much fibrous, cartilaginous, osteoid, or osseous stroma, diminution in size cannot be expected as a sign of radium effect, and cessation of growth followed by a long period of gradual shrinkage is all that should be looked for.

In a bulky carcinoma of the lung, the radium pack caused extensive necrosis of most of the tumor, but an invasion of the spinal column destroyed a vertebral body and led to paraplegia. Protected by the bony tissue, the invading tumor cells appeared to be entirely unaffected by the radium.

In general it may be said that, in dealing with bulky deep seated tumors, the result will be strictly dependent upon the structure of the growth and its accessibility to radium, but that the clinical outcome will not always accord with the amount of influence exerted on the tumor. I feel disposed to recommend that such deep seated tumors be treated rather cautiously.

Arteriosclerosis is very frequently observed in the neighborhood of carcinoma of the skin or mucous membrane, and, indeed, arteriosclerosis has been urged by many as a fundamental predisposing condition to cancer in these tissues. Its probable existence should be taken into consideration in attempting to treat any well established carcinoma of a mucous membrane, especially in elderly subjects, by heavily filtered radium. For radium itself tends to produce a slow and progressive obliterating endarteritis, the effects of which may not be seen for weeks or months after the exposure. The results of such endarteritis may be late sloughing, or the production of an indolent ulcer which refuses to heal although most or all of the cancer tissue has been destroyed. In such cases, the use of small amounts of unfiltered radium, the radius of action of which is superficial and restricted, should be considered, and in general, I believe, preferred.

Many workers with heavily filtered radium have observed that with each repeated dose the regressive effects in the tumor are less and less definite. It has even appeared at times that the tumor tissue became very resistant to the radium, while the normal tissue, especially the stroma, became less resistant. In a case of basal cell carcinoma of the scalp, repeatedly excised and returning each time over a wider area, then treated with the *x*-ray and finally by repeated doses of filtered radium without satisfactory results, I found on the edges of the ulcer apparently living and well staining tumor cells, lying in a hyaline or necrosing connective tissue entirely devoid of circulation and quite incapable of any effort at healing. It is not possible in this case to say that the tumor cells were growing; the lesion was not advancing clinically, but it is certain that no important therapeutic result had been secured by the radium.

To what extent this reversal of the relative susceptibilities of tumor tissue and normal tissue occurs after the use of repeated doses of heavily filtered radium, I am unable to state, but I believe that it represents a definite limitation of repeated treatments by the gamma ray.

An important therapeutic use of heavily filtered radium which is probably inadequately recognized, but which has been pointed out by Levin for the *x*-ray, consists in the retardation of the rate of growth of the tumor cells and the transformation of an active malignant into a

slowly growing or quiescent and relatively benign tumor. In dealing with very advanced epidermoid carcinoma, or active adenocarcinoma which has advanced beyond the area which can effectively be reached by radium, most observers realize that the object of treatment should be palliative and not curative. Under such circumstances the use of heavily filtered radium over long periods at a distance from the skin may often bring the tumor to a standstill, relieve pain, reduce pressure, permit the patient to take on weight and prolong life without inflicting any perceivable injury or discomfort. It is in this field that radium has proved of inestimable value as a palliative, whereas more aggressive attempts to eradicate completely the disease, in those deplorable post-operative recurrences which are constantly being thrust upon the hands of the radium worker, often end disastrously.

Among the interesting results of this type which I have observed is one in which an originally active and cellular epidermoid carcinoma of the lip, metastatic in the lymph-nodes, had been transformed into a structure replacing the nodes and resembling a sebaceous cyst lined by normal skin. In another case, an epidermoid carcinoma that was invading the labial artery had been transformed into a structure resembling normal epidermis. Such transformations are occasionally seen to arise spontaneously in the course of epidermoid carcinoma, but they are very frequently seen in cases treated by radium.

It is a general experience in radium clinics that some cases of epidermoid carcinoma respond badly to radium treatment and after an initial inhibition of growth seem to progress very rapidly, even more rapidly than before, so that the question of an acceleration of growth has arisen. This explanation has been accepted by those inclined to do so. In several such cases, I have been able to demonstrate a satisfactory cause for the unfavorable course of the tumor and the rapid decline of the patient, consisting in the presence of active tuberculosis of the lungs and other organs, with tuberculous infection of the tumor; while in other cases, the patient was suffering from advanced tertiary syphilis of the organs and of the tissues and vessels in the vicinity of the tumor. While in these latter cases syphilis was suspected from the clinical signs and a positive Wassermann, the tuberculous complication in the former was overlooked in several instances.

These observations merely emphasize the fact that in treating carcinoma by means of radium, or of any other agent, we are not always dealing with a perfectly healthy body nor with an uncomplicated local tumor, and that a broad general clinical point of view must guide the therapeutist in the use of such a powerful agent as radium.

In a previous communication, based largely on the clinical work of my colleagues, I expressed the view that the constitutional action of large doses of heavily filtered radium was not a serious fault in its use and that such constitutional action, if it occurred, was largely referable to active necrosis of tumor tissue and to infection. Further experience confirms this opinion, since it is often shown that even rather large tumors may be rapidly absorbed without constitutional disturbance.

On the other hand, when the tumor is infected, especially in epidermoid carcinoma, the use of radium in any form may be followed by aggravation of the infection with spreading phlegmonous inflammation, erysipelas, pneumonia, or septicemia. Fortunately, these serious complications are rare, but the possibility of their occurrence must be kept in mind in any form of radium treatment of an advanced and infected malignant tumor.

Even this brief catalogue of some of the things which radium can not accomplish with advanced carcinoma, and especially with post-operative recurrences, inclines me more and more to reaffirm the position taken two years ago, that the most successful and, accordingly the proper field for radium, will be found in strictly localized and therefore operable carcinoma.

DISCUSSION

Dr. H. Gideon Wells (Chicago): Dr. Ewing suggested that arteriosclerosis follows the destruction of tissues on account of the diminished volume of blood passing through the vessels. This, in itself, might perhaps account for a great deal of the arteriosclerosis that is seen in such cases.

Dr. F. C. Wood (New York): Dr. Ewing has brought up many interesting points, for instance, alteration in the type of a tumor. I have been receiving material from a colleague who treats many tumors about the face, and find that I get for examination only those which have resisted a considerable quantity of radium. Basal-cell tumors which contain keratin seem able to resist radium, and I have been endeavoring to get material before the tumor is treated, to see whether radium kills cells of the basal type and spares squamous elements, or whether the basal cells differentiate into squamous cells under radium treatment; in other words, whether this is another example of the change of type discussed by Dr. Ewing.

Radium toxicity is also interesting. I have seen one or two cases of this condition, but without extensive changes in the tumor, or resorption, such as Dr. Ewing has observed. I have wondered whether it is not due, in some cases at least, to progressive destruction of the bone marrow, or to an alteration in the function of the liver, brought about by radiation which has escaped. We do not always remember, in employing radium, that more or less remote regions also come under its influence. The anemia which so often occurs in leukemic patients that are receiving treatments over the spleen, indicates the wide influence of radiation. The shrinkage that takes place in the spleen within a few days in these leukemic cases, brings up the question why these enormous splenic tumors should disappear without causing toxic symptoms. I have not seen a severe toxemia after radium in any case of myelogenous leukemia.

A third point in Dr. Ewing's remarks is the question of a development of resistance to radium under certain conditions. Dr. Frederick Prime and I are engaged in a series of experiments which we hope will show whether or not resistance supervenes in cases in which tumor cells are transplanted through a series of animals. We radiated a tumor, transplanted it, radiated it again, and transplanted it again, in order to see whether there was any difference in the number of takes and in the rate of growth. We found no difference. My theory is that after most of the carcinoma has been killed by radium, the few remaining cells at the periphery have sufficient nutrition to take care of themselves, being at a site where the vascular supply is abundant.

The lethal dose for cells *in vitro* is only half of that necessary *in vivo*. That is, to kill a cancer cell where there is no circulation requires about half the radiation requisite where the circulation is still in full force; whatever the products are that destroy the cells, they appear to be swept away by the circulation.

4. Effect of *x*-Ray on Tumors

Dr. Francis C. Wood and *Dr. Frederick Prime* (New York):

SUMMARY

While much has been done with the *x*-ray of late, the lethal dose for tumor cells under what may be called the usual conditions employed in practical work has not yet been accurately determined. Nevertheless, such a lethal dose must be determined if *x*-rays are to be intelligently used in the treatment of internal malignant growths. The conditions of superficial treatment, on the other hand, are well enough known.

In order to determine this lethal dose, therefore, the following experiments were carried out.

As with previous experiments in which radium instead of *x*-ray was employed, the tumors used were a mouse sarcoma (Crocker Fund No 180) and a mouse carcinoma (Crocker Fund 11); mouse embryo kidney was also used. The material was removed under aseptic precautions and cut into small pieces, about 0.001 gram in size. These were moistened with a drop of Ringer's solution and put into hollow slides, which were then covered with cover-glasses and sealed.

About twelve minutes were required to produce an erythema reaction with the *x*-ray apparatus employed, at a distance of 9 inches from the anticathode of a Coolidge tube with 3 mm. of aluminium as a filter, when 5 milliamperes of current were used with an 8.5 inch spark gap. The tissue was treated in gradually increasing doses ranging from six to forty-five minutes.

Some of the treated tissue was then inoculated into mice and the rest was planted in plasma. The results were as follows:

Where sarcoma was inoculated at once into mice after treatment with x-ray, there was no difference in the number of takes or in the rate of growth between controls and treated tissue, until the tumor had been given x-ray for twenty-five minutes; from this point on, there was a decrease in the number of takes and the rate of growth, until at forty-five minutes there were practically no takes.

The tumor which had been radiumized and planted in plasma, however, showed most profuse growth even at the end of forty-five minutes. This growth was photographed for record and the plasma plants then inoculated into mice, where they grew in practically the same manner as did the tissue which had not been planted in plasma.

With mouse embryo kidney the same profuse growth was shown in tissue that had been given x-ray up to forty-five minutes, but no controls could be made by inoculating into animals.

Mouse carcinoma was subjected to the same treatment, with a slightly different result. There was never such profuse growth as was seen in the sarcoma and kidney tissues, and the time required to slow the rate of growth was less, since after an exposure of twenty-five minutes there was no growth after inoculating the tissue into mice, though there was still a slight *in vitro* growth when pieces of x-rayed tumor were planted in plasma.

Another series of experiments was undertaken, in which mice with tumors were treated with x-ray, after which the tumor was removed and inoculated. In these experiments the mice were put in a small box and treated for from ten to sixty minutes. At the end of forty minutes the transplanted tumors began to show some slowing, but even after being treated for sixty minutes there was good growth at the end of two weeks in about 50 per cent of the mice inoculated.

While these experiments are not yet in shape for final report, it is evident that about twice as much radiation is required to kill tumor cells *in vivo* as *in vitro*. In other words, seven to eight erythema doses must be applied at a single sitting to kill cancer cells.

DISCUSSION

Dr. Ewing: I should judge that the dose that Dr. Prime uses exceeds the dose that one attempts to employ in therapeutics for the treatment of a primary tumor, for the control of metastasis, or in postoperative radiation. If that be so, the results would indicate that any reasonable amount of x-ray treatment in these circumstances is without experimental basis; yet clinical experience shows pretty definitely that human tumors will regress rapidly, at times, after the application of a much smaller amount of x-ray than Dr. Prime is using, if my estimate of the dosage he employs be correct. In other words, the results of different investigators are diametrically opposite. Is there any explanation for this? I think there is one that should not be lost sight of. Dr. Prime is estimating the effect of rays on tumor cells only;

but these are not the only elements concerned in therapeutic experience. Surrounding cells in the body are also affected, as histological studies show. Thus in the regression of epidermoid carcinoma I have found tumor cells rapidly disappearing by a process of growth of granulation tissue; and I drew the conclusion that recession is the result of this proliferation of the stroma, and not of the radiation itself.

Again, the question of the amount of growth actually taking place *in vitro* must not be lost sight of. Whether the separation of cells from the main parent mass can be interpreted wholly as due to growth, or whether it be partly an expression of other physical changes in the parent mass, is, I think, open to question. The better test is the biological test: What is the result of transplanting the tumor cells back into the animal? If they grow, we are sure that they have not been killed; yet even this is not a complete duplicate of therapeutic experiments. Nevertheless, I welcome this type of investigation, and believe it important that the activities of the radium therapists be supported by this sort of experiment.

Dr. Prime: I think that perhaps Dr. Ewing does not realize how hard it is to duplicate therapeutic doses in the laboratory. The slides were exhibited in order to show that even forty-five minutes exposure does not kill. We obtained exactly the same result with radium.

Dr. Wood: This work is part of a general experiment, outlined to discover whether there is any difference between the cancer cell and the normal cell; and if there be, to determine what it is. The reason that tissue cultures were employed is that the most convenient method at present of demonstrating life is to show that the cell will grow; vital stains, also, may occasionally be of service. Furthermore, tissue cultures permit a study of the effect of radiation on cells entirely isolated from the body as Dr. Ewing has pointed out, so that the conditions of the experiment are as simple as they can be made. We have endeavored, in all other respects, to reproduce the conditions under which the clinician employs the x-ray. The filter ordinarily chosen by the practitioner is one that will permit an attack of the deeper tissue, and we accordingly used one of three millimeters, such as he usually employs.

Our figures were obtained from tumors of the highest virulence. A cell from a basal-cell epithelioma is probably twenty times as susceptible to x-ray as one from a mouse tumor, but I do not doubt that there is a type of human neoplasm that is as resistant as any mouse tumor. We must conclude that to kill a tumor cell just beneath the skin it is necessary to use eight times the erythema dose at a single sitting. To kill the ovarian follicles requires five hours' exposure, and a tumor cell is still more resistant. The necessity for this enormous dose is due to the increased distance from the tube, and to the absorptive power of the tissue, 8 cm. of which remove about two-thirds of the energy of the rays employed. Obviously such doses are clinically impossible. We are trying to work out a method of treatment that the

practitioner can apply, and to do this we must study the various susceptibilities of human tumors. The curious thing is that these experiments show the cancer cell to be more resistant than the skin, since six or eight erythema doses are required to destroy it.

The dividing cell is known to be three times as sensitive to radium as others, and variability in the number of takes is perhaps due to the stage of division that the majority of cells in a tumor are in; in one growth, most of them may have just passed through mitosis, while in another the majority may be in a pre-mitotic or mitotic phase.

The percentage of takes shows fluctuation in the material, it is true; but one single take proves that that tumor would have recurred in a human being.

Dr. W. T. Bovie (Boston): It might be possible to get a differentiation that one could see under the microscope, by raising the temperature of the cells above normal. In some experiments that I expect to describe more fully this afternoon, I found that I can radiate paramecium with ultraviolet light without producing any morphological change, until the temperature is raised to 24°C. At this point, the protoplasm promptly cytolyzes.

Dr. William Duane (Boston): May I ask what the temperature was?

Dr. Prime: 37° in the incubator.

Dr. Duane: There is a surprising difference in *x*-ray radiation when the temperature is changed. Penetration increases also with the voltage; doubling the voltage produces eight times the penetration. Measurement of the voltage by the spark-gap is not very accurate for it gives no information about the intermediate voltage. It may be that the intermediate voltage between these points is very small. This should be taken into account when experiments made with one machine in one place are compared with other experiments made with a different machine in another place.

Dr. Wood: That is true. But we found accurate measurement a waste of time so far as our purpose was concerned, which was merely to get practicable results for the use of the clinician. I fully realize, however, that there is a wide difference in apparatus, for one of my assistants has a duplicate machine, installed within a few weeks of ours at the Crocker Fund, and actually adjusted by the same man; yet the two give entirely different results, as determined by the photographic or the pastille method.

Dr. Ewing: In 1913, in Paris, Keating-Hart was working with an *x*-ray apparatus with which he said he was curing advanced carcinoma of the skin. He said, "You cannot cure it with the *x*-ray, unless you

raise the temperature;" and he did so, putting one electrode under the patient's body and connecting it with aluminum foil, and raising the temperature until the patient cried out from the heat.

5. The Comparative Pathology of Tumors of the Testis

Dr. Maud Slye, Miss Harriet F. Holmes, and *Dr. H. Gideon Wells* (Chicago):

SUMMARY

New growths of the testis are common in horses and dogs, exhibiting quite the same characteristics as corresponding tumors in man. But they seem to be very rare in all other species, and no mention of such a tumor in the mouse can be found in the literature. In the Slye stock, among 19,000 mice dying natural deaths and examined post mortem, about one-half of which were males, 28 instances of primary growth of the testis were found. Most of these resembled in all essential features the neoplasms that arise in the testis of man and other animals, consisting of cells closely resembling the epithelium of the seminiferous tubules and often arranged in an alveolar structure. Despite great vascularity and a distinctly atypical structure, no remote metastasis was observed, although in one case a series of six contiguous independent nodules was formed, and one had tumors in both testes. Two of the cases seemed to be true spindle-cell sarcoma, one arising at the site of a wound. Three of the typical "orchidoblastomas" also followed trauma. No evidence could be obtained that any of these tumors had arisen in a teratomatous growth, and no cases of teratoma have been observed.

One case of spindle-cell sarcoma of the seminal vesicle of a mouse is described, apparently the second case of a tumor of this organ reported as occurring in a lower animal.

Two cases of primary spontaneous tumor of the testis in dogs are described.

With the exception of one sarcoma, all the 28 new growths of the mouse testis occurred in the members of a single strain of mice and its hybrid derivatives, thus substantiating the statement that heredity influences the incidence of tumor development in different organs or tissues. This fact also probably explains the absence of any recorded cases of tumor of the testis in mice from other laboratories.

DISCUSSION

Dr. Woglom: The paper of Dr. Wells has great interest for me, because for the past three years or so I have been trying to produce tumors in mice. There are three probable causes of cancer so far known—age, hereditary predisposition, and perhaps some form of chronic irritation. Since in a mouse with a spontaneous new growth

two of these are already present, only such mice have been employed. Yet although various sorts of irritation have been applied, including tar, as recently described by two Japanese investigators, no tumor has been produced. Some of Dr. Wells' growths followed trauma, and it occurs to me that these mice may have lived longer than mine, none of which survived much more than five or six months, since they are already middle-aged when they reach the laboratory. I should be greatly obliged to Dr. Wells, if he would tell me how long an interval elapses between the injury and the tumor.

Dr. Leo Loeb (St. Louis): Several years ago we began some experiments in which we applied long-continued stimulation of various tissues to certain mice in which we had previously established a considerable predisposition to cancer. Both theoretical consideration and well-known facts had suggested to us that hereditary predisposition and external or internal stimuli (internal secretions) are each of importance in the etiology of cancer—a view that we have expressed on various occasions. We applied tar to the skin of our mice, or we caused chronic ulceration in other ways; in some cases, again, we pulled a thread through the mammary gland. But so far our experiments have all been negative, no cancer having been produced in this way.

Dr. Wells: I cannot answer Dr. Woglom's question how long after the trauma the tumor developed, because Miss Slye has the records. The system on which we have done the work was to keep the one end as far from the other as possible. I did not know the heredity, and she did not know the diagnosis, until we brought our parts of the work together.

6. The Effect of Heat upon Tumor Tissue

Dr. Holland N. Stevenson (New York):

SUMMARY

The effect of heat upon tumor tissue has been studied by several investigators and some interesting points in regard to the general biological properties of tumor cells have been determined. The work of Loeb stands out prominently. This author, using large numbers of animals, demonstrated the progressive inhibition of the growth power of a mouse carcinoma when kept at 44°C. for from five to sixty minutes. He found that the latent period of growth and the number of regressing tumors increased with the time of exposure to the heat, and demonstrated that these effects were not transferred, in any striking degree, to subsequent generations. Lambert later showed that tumor was more susceptible to heat than adult connective tissue, when both were growing *in vitro*. Haaland was able to eliminate the carcinomatous portion of a carcinosarcoma by exposure to heat, an indication that the carcinoma cells were more susceptible to heat than sarcoma

cells. Various other investigators (Clowes, Clowes and Baeslack, and Michaelis) have also described the effect of heat upon tumors.

The object of the experiments here reported was to establish accurately the lethal points for tumor exposed to a series of temperatures for varying lengths of time, so that the death points could be used as a basis for further work with normal tissues. Another object was to determine, if possible, the curve that these points would make if plotted according to the factors of temperature and time of exposure. It was also of interest to determine whether or not carcinoma cells were actually much more susceptible to the influence of heat than sarcoma or normal epithelial and connective tissue cells.

The tumors used were the Crocker mouse carcinoma 11, and mouse sarcoma 180. These have a growth rate approximately the same, although the latter grows a little more rapidly and has a little higher percentage of takes. The tumors were cut into small pieces (0.002 grams), of which 24 were placed each in a small test tube containing about 1 cc. of Ringer's solution. The tubes were then placed in a large Wassermann water bath, of such size that temperature fluctuations due to radiation were reduced to a minimum; control pieces were placed in the ice-box. The temperature of the water bath was registered on thermometers reading to 0.01°C. and checked by delicate certified thermometers. At definite periods, readings of the thermometer were recorded and a tube containing a fragment of tumor was removed; the tumor was then cooled and inoculated into 24 mice, control tumor from the ice-box being inoculated at the same time.

The results showed that the death points of the tumors fall upon a curve which must be made rather broad in order to take in all the deviations encountered. These deviations must be due to different biological conditions of cells in the same tumor particle which it was impossible to control (e.g., mitosis, etc.), and to the different tumors used, rather than to temperature or other physical variations.

At 39.5 to 40.5°C., the sarcoma stopped growing after two and one-half hours' exposure. The carcinoma showed no growth after two hours, except that one stray tumor developed at four hours.

At 42.5° the sarcoma stopped growing after forty-five minutes' exposure, while at 44° it did not grow after one hour, except that two stray tumors developed after two hours' exposure. The carcinoma was found to stop growing after forty-five minutes at 44°.

At 47.75° to 48.25° no growth of either sarcoma or carcinoma occurred after fifteen minutes' exposure.

A review of these results shows that the curve for each tumor is broad, and not limited to such a definite line as a rectangular hyperbola. This is especially indicated by the occurrence of tumors (sarcoma) at 44° after one hour exposure when at a lower temperature (42.5°) they do not grow after less exposure, as well as by the development of tumors from one or two grafts after an exposure long enough to kill tumor cells in other cases.

Neglecting those tumors which appeared after a time of exposure far beyond the period when the other portion of the tissue had been killed, and considering as the death point the time of exposure at a certain temperature when no tumors developed, it was found that through these points a curve could be passed that would be approximately at a minimal distance from them. This curve was a simple hyperbola, plotted according to the formula $XY = K$, with K in this instance arbitrarily equal to 15. If the factor K be made equal to 8.8, and again to 20, two curves are obtained on or within which all the determined death points fall.

These points were plotted on the rectangular coordinates Xy, X being the time of exposure and y the temperature. The zero of temperature was taken as 39°C. the approximate body temperature of the animal, for it was considered that at this temperature the tumor tissue would live indefinitely; or if $y = O$ than $x = \infty$

From the results obtained, no difference between sarcoma or carcinoma in their susceptibility to heat can be made out. The experiments are to be continued to include normal epithelium and connective tissue.

DISCUSSION

Dr. Wells: I should like to ask Dr. Stevenson whether he attempted to find, in the few cases where tumors grew after exposure to the normal death point, whether any difference in thermo-resistance appeared in subsequent generations.

Dr. G. H. A. Clowes (Buffalo): I should like to ask Dr. Stevenson whether there was any evidence of a stimulation of growth. We observed this in a few cases, but were unable to reproduce the phenomenon in a later attempt.

Dr. Loeb: I would ask Dr. Stevenson whether there has been noticeable, in any case, an increase in the duration of the latent period after the transplantation of heated tumors.

Dr. Stevenson: In reply to Dr. Wells, I would say that no attempt has yet been made to establish a thermo-resistant tumor.

Neither retardation nor stimulation could be demonstrated.

With reference to an increase in the duration of the latent period, it may be said that this was not constant enough to be of any value. The number of instances in which the latent period was increased was not sufficient to indicate anything definite.

Dr. Wood: Here, again, I think that fluctuations mean differences in the composition of the biological material. The number of cells that have divided is uneven in samples obtained by selecting pieces of a tumor.

7. Further Studies on the Importance of the Lymphocyte in Cancer Immunity

Dr. M. J. Sittenfield (New York):

SUMMARY

These experiments are supplementary to those reported before this Association last year, and corroborate them entirely.

The experiments of Murphy and Morton with mice were repeated with the Flexner-Jobling rat carcinoma, but no relation between the lymphocyte and immunity to transplanted tumors could be demonstrated.

DISCUSSION

Dr. Wells: We have frequently observed that spontaneous tumors develop in mice with lymphatic or myelogenous leukemia, as they do in other mice; and that even where there is a prodigious increase in the lymphocytes, the tumor is entirely unaffected.

Dr. Loeb: Might it not be that two factors are necessary for the action of the lymphocyte? Lymphocytes must be present in sufficient numbers, and the path to the tumor must be free. The tissue must exert an attraction on the lymphocytes, and it is this latter factor that is especially important.

In the case of homoiotransplantation, lymphocytes and connective tissue are very important factors in the destruction of tissues, but this applies only to certain varieties. With others, and in heterotransplantation, the lymphocyte plays a minor part, and the incompatibility of the body fluids suffices to destroy the graft.

Dr. H. T. Marshall (Virginia): In cancers and other pathological conditions it is often difficult to escape the impression that lymphocytes are in some way involved in the reaction to the pathological condition. In a case of lymphadenoma which I hope to report tomorrow there are certain appearances which, it seems to me, are most satisfactorily accounted for on the assumption that the lymphocytes are engaged in some form of protective reaction.

Dr. Wood: An interesting fact, from the morphological point of view, is the extreme facility with which carcinoma grows in the lymph-nodes in the human body. Again, we have at the Crocker Fund a guinea-pig sarcoma which takes in but few animals and grows very slowly. According to the hypothesis advanced by some investigators, reduction of the lymphocytes would facilitate the growth of this tumor; yet it exerted no effect at all. Finally, we have inoculated a leukemic mouse with a mouse tumor, and found that it grew as well as in a normal one.

Dr. Loeb: The great significance of the lymphocytes in the destruction of grafted tissues sometimes comes out very strikingly after the transplantation of thyroid from mother to child or from sister to brother. In such cases, transplants are often so perfectly preserved as almost to resemble autotransplants; but in most cases lymphocytes gradually begin to appear in large masses and to destroy the transplanted thyroid. We have every reason to assume that without the appearance of lymphocytes the tissue would have remained alive much longer.

Dr. Wood: How can you tell whether the lymphocytes appear as scavengers after the tissue has died, or whether they themselves destroy?

Dr. Loeb: Lymphocytes appear among acini that look perfectly well preserved, resembling those in a normal thyroid that has not been transplanted. Indeed the graft which is thus attacked often shows mitotic proliferation. The lymphocytes then actually invade these acini, and overwhelm and destroy them, the destruction occurring first where the lymphocytes appear first in largest number—that is, around the larger vessels. In heterotransplantation, tissues perish without the intervention of lymphocytes. Unstriated muscle dies sooner or later after homoiotransplantation, but lymphocytes are not often attracted in large number. Dying tissue does not, as such, attract lymphocytes; but certain metabolically abnormal tissue products, such as develop in epidermis and certain glands after homoiotransplantation, have this effect. These metabolic abnormalities, as such, would be compatible with a longer duration of life, were it not for the activity of the lymphocyte (and the connective tissues).

Dr. Wells: Surgeons say that the best place to transplant thyroid is in the bone-marrow and the spleen.

Dr. Loeb: That does not contradict what I have been saying; the essential factor is that there be some product in the cells which attracts the lymphocytes. The attraction is exerted upon the lymphocyte under certain conditions by the products of tissue metabolism.

Dr. Wells: On that account, it must be primary in the cell.

Dr. Loeb: Yes; it is the two factors combined. The stimulus plus the cell. If either be missing, the result is not forthcoming. However, there can be no doubt (and in this respect I agree with the opinion expressed here, which I myself have upheld on previous occasions) that lymphocytes play a part in destruction of tissue and tumor grafts only under certain conditions. Other factors are in play beyond question.

Dr. Ewing: I wish to speak of a matter which was mentioned last year in the discussion; I refer to the experience of the general pathologist at the autopsy table. I have long since given up trying to demonstrate any relation between the activity of the lymphoid apparatus in the human body and the growth of cancer, and I believe that it is impossible to do so. Actively growing tumors are found in young subjects with lymphoid tissue in great abundance; on the other hand, widespread neoplasia involves the bodies of elderly subjects, in whom the lymphoid apparatus is deficient. The same observation applies to the size of the spleen; I have been quite unable to discover any relation whatsoever between the rapidity of growth and metastasizing power of different tumors, and the size of the spleen. Hence I do not believe that any information can be drawn from postmortem experience to favor the theory that any part of tumor immunity is due to the lymphatic apparatus.

Dr. Sittenfield: So many have defended me that very little remains to be said, except, perhaps, to supplement their statements by recalling the fact that malignant lymphomata occur in lymphoid tissue only

8. Experimental "Carcinomata" of Animals and Their Relation to True Malignant Tumors

Dr. F. D. Bullock and *Dr. G. L. Rohdenburg* (New York):

SUMMARY

None of the publications describing the experimental production of new growths can be accepted without reserve. Against them are the facts that the tumors are usually said to have been produced in young animals as readily as in old; that they generally do not continue their growth after the irritant has been discontinued; that they are not transplantable; and that no really reliable evidence of metastasis has been offered. The lesions were probably hyperregenerative processes.

A full description of a long series of unsuccessful attempts to produce tumors will be published at some future time.

DISCUSSION

Dr. Wells: I had recently the opportunity of visiting Professor Yamagiwa, and spent a large part of the day in going over the material at his laboratory. It is true that many of the simple regenerative lesions described here to-day appear in his material, but I saw specimens in which proliferation had continued after the application of tar had been stopped, causing widespread ulceration and reproducing histologically as perfect a carcinoma as I have ever seen; there were even metastases in the lymph-nodes, one of them 2 mm. in diameter. In some cases in which the irritant had been removed, ulceration had

continued for months, progressively destroying the tissues. After examining his material, I could see no reason to doubt that squamous-cell epithelioma had been produced by protracted chemical irritation.

I might add that a gastric cancer in a mouse, which I described at last year's meeting, was associated with a hair ball, and thus forms an interesting parallel to those experiments of Dr. Rohdenburg in which he irritated the gastric mucosa by introducing spinous balls into the stomach.

Dr. Loeb: I agree with Dr. Rohdenburg that a critical attitude regarding purely morphological criteria is necessary, where questions of the transformation of normal into cancerous tissue are concerned. However, there can be no doubt that such a transformation does certainly occur under the influence of long-continued stimulation; for instance, in those numerous cases in which a Roentgen ray dermatitis becomes gradually transformed into cancer. But the important point would be to determine when the biological change takes place; purely morphological criteria are often indecisive.

Dr. Ewing: It seems to me that between hyperregenerative overgrowth and true neoplastic hyperplasia, there is a distinction without any difference. I myself would conclude, not only from previous work but also from the startling results that Dr. Rohdenburg has presented here to-day, that he is describing lesions similar to or identical with the early stages of developing carcinoma in man. If he is going to assume that there is a distinction, he must have something more definite than mere position of cells in the tissues to support his contention. Dr. Loeb has referred to the fact that x-ray burns often become carcinomatous; yet these closely resemble the lesions just exhibited by Dr. Rohdenburg. The altered cells are strictly confined within the malpighian layer, and there is not a hint of danger to the patient. Nevertheless, and almost without exception, these cases of chronic x-ray dermatitis suddenly develop carcinoma. When the cells do break loose, progress is rapid.

I am impressed by the fact that islands of epidermization may be found in the lungs of rats; the matter was emphasized at Paris in 1913. At the same time, however, I cannot see that even though such a lesion is known to occur independently of carcinoma, we are justified in discarding the lesions which Fibiger has described.

While it seems wise to me, on the whole, not to be too hasty in drawing conclusions from purely histological data, I believe, nevertheless, that in the complete series of transitional stages which have been discussed here this morning, we are dealing with the beginnings of carcinoma.

Dr. Wood: It seems to me that Dr. Ewing has put his finger on the question; and also that the difference between true carcinoma and Dr. Rohdenburg's lesions, which he judged to be mere proliferations be-

cause they disappeared when the irritant was removed, is what brings us here this morning. Why a cell, whatever its position or morphology, should suddenly do something that a normal cell does not do is the problem upon which we are working.

Dr. Rohdenburg: I am, of course, at a disadvantage in having seen only the illustrations of Yamagiwa and Ichikawa, and not their original preparations. I agree with much that Dr. Ewing has said, and merely desired to point out the frequent danger of diagnosing malignancy solely on the ground of histology.

In order to produce a tumor it is probable that beside the irritant, some sort of tissue predisposition, as well as age, is required; and I am convinced that too little attention has been paid by experimentalists in the past to the factor last named.

Dr. Ewing: Is it not true that tumors in the lower animals are slow to metastasize—that the tissues of lower animals are not so favorable for metastasis as those of the human being? If so, we have no right to demand metastasis to the same extent in the lower animals that we do in man.

Dr. Wood: I believe that this is due merely to anatomical conditions. The lymphatic system in the lower animals is, perhaps, not so well developed, and that may be why metastasis in the lymph-nodes is rather uncommon in mice.

Dr. Wells: I used to accept the anatomical explanation for absence of metastasis until there came under my observation a strain of mice which often developed carcinoma of the liver. When we came to cast up the cases of metastatic tumors in the liver, we were astonished to find that nearly all of them occurred in this strain. It seemed obvious that the location of metastases was not, therefore, so much a matter of anatomy as had been supposed, but that some sort of tissue predisposition might be involved. We know, too, that the lungs of patients with gastric carcinoma are often full of emboli in all stages of healing, and we have good evidence of emboli passing through the lungs without settling there. And only recently I saw at autopsy a case of extensive carcinoma of the thyroid gland, with both adrenals, both mammary glands, and both ovaries extensively involved by metastasis. Such findings as these cannot be explained on an anatomical basis.

Dr. Loeb: I have had occasion previously to indicate that there is a great deal of evidence in favor of the conclusion that while both hereditary predisposition and external or internal stimulus are important for the origin of cancer, a certain reciprocal relation between these two sets of factors exists. If there be a powerful stimulus the amount of predisposition requisite is less; and it is very probable that in certain cases in which the stimulus has been unusually potent predisposition be-

comes almost or quite negligible. Thus it has come about that many roentgenologists who, in earlier years, did not know how to protect themselves, developed cancer; we cannot assume that they all happened to be predisposed. It is similar with the factor of age, which is important in animals as well as in man. Among cattle, for instance, cancer usually attacks old individuals, as I pointed out in 1899; yet old age is not an absolutely necessary factor. Malignant tumors may occur in young persons, and even in children; and in mice cancer of the mammary gland appears not rarely in relatively young animals, only six or seven months old. Age is therefore probably not a direct factor, and may merely be of significance in that it permits stimulation to extend over a long period of time. How far structural senile changes, as such, predispose to cancer, is unknown.

Dr. Wells: So far as the spontaneous tumors of mice are concerned, the age curve corresponds exactly with that in man. New growths appear in the human subject with increasing frequency from the twentieth year onward, and in mice at a corresponding period, i.e., from six months onward. I do not see, therefore, how age can be dismissed as a factor of no importance.

Dr. Loeb: I did not mean to say that age and predisposition are not important; merely that they do not seem to be indispensable. Cancer may appear in relatively young persons, if irritation has been applied for a sufficiently long period.

Dr. Rohdenburg: Regarding the length of time that it is necessary for an irritant to act, I would say that we have been investigating a tape-worm that appears to be largely responsible for sarcoma of the liver in rats. We have found that this grows at the rate of 0.5 cm. a month, and that the length of those associated with sarcomata proves they had been present for a year. This is a long period in a rat's life, as compared with that of a human being.

Dr. Clowes: There has been a great deal of discussion of the question of border-line cases, and I should like to know whether the speakers have made use of a simple method that has recently been devised for determining the permeability of the tissues concerned. We know that various toxic agents and chemicals can induce an increase in the permeability of sea-urchin's eggs, etc. If the damage be carried only to a certain point, recovery may be brought about by changing the environment; but if carried beyond this point, the damage cannot be recovered from. A similar experiment might be carried out on human or animal material, giving the investigator something definite and concrete to lay hold of.

Mr. M. C. Marsh (Buffalo): We have seen at the State Laboratory in Buffalo one female mouse with a typical mammary tumor arising at

the age of three months. The other mice of the strain, which has a high incidence, produce tumors at the usual time, after the age of six months.

Dr. Wood: One point was brought up by Dr. Wells to which I might contribute a few notes; I refer to the susceptibility of organs to metastasis. There has been a great deal of discussion on the inadvisability of removing a piece of tumor for purposes of diagnosis and there are, in fact, two schools. One prefers to excise a fragment for diagnosis, if necessary, while the other would extirpate a tumor on the clinical evidence alone, believing that in doubtful cases it is better for the patient that the growth be removed, even though it should ultimately prove not to have been malignant. The discussion has been rather acidulous, especially in New York City, where there has appeared a series of editorials on the subject. Dr. Bloodgood, of Baltimore, chief opponent of excision for diagnosis, says that he has no record of cure in any case where a portion was removed for diagnostic purposes. On the other hand, those tumors that I have seen cured at St. Luke's Hospital, in New York, have been the very ones from which a fragment had been extirpated for diagnosis.

We have carried out some experiments with the Flexner carcinoma on 228 rats. We found no more metastases in rats that had had pieces removed from their tumors than we found in the controls.

We were surprised to find, however, that there was a distinct difference (as high as 50 per cent) in the percentage of lung metastases in rats from three different dealers. This illustrates two points; First, the remarks of Dr. Wells concerning organ liability to metastasis. Secondly, it explains the frequent differences in the percentage of metastases reported by different investigators who have worked with this tumor.

Dr. Ewing: This is an important question, and I am glad that it has been brought up in this society, supported by experimental data. The problem has been attacked by clinicians, though with a great variation of opinion. Dr. Park and I are acutely interested in the matter, because I was responsible to a considerable degree for the introduction of a diagnostic service by the Health Department of New York City. I believe thoroughly in the wisdom of that move, under proper restrictions. My main objection to the conclusions of clinical observers is the one mentioned by Dr. Wood: they have drawn sweeping conclusions from limited personal experience, and under conditions which I believe to be wholly unscientific. Thus, they recommend almost without exception a cauterization of the wound made in removing the tissue. But I doubt whether the considerable approval with which the clinicians view this prophylactic procedure can be endorsed from the laboratory standpoint. I myself think that it is an undesirable addition to the damage already done to the tissue by the scalpel, and I would suggest to Dr. Wood, if I may, that it would be a useful

enlargement of his experiment to investigate the effect of this additional damage. I believe that after a clean incision into a tumor, without pressure or trauma, there is very little chance of spreading the disease, whereas the cautery produces a notable hyperemia over a wide area which almost certainly would increase the growth of the tumor itself, and, as I visualize the situation histologically, would increase the tendency to loosen the tumor cells.

One has to admit that the excision of a portion of any tumor for microscopic diagnosis is, after all, a confession of ignorance, and we deplore the fact that it is often impossible to determine the nature of a tumor in any other way; hence our procedure must be determined entirely by the danger to the patient brought about by diagnostic excision, on the one hand, and the greater danger of a wrong diagnosis on the other. We may be able, at some time in the future, to diagnose cancer without histological examination, but until that time comes, I, for one, would rather have my tongue cut out by someone who had proved that a cancer was present, than by one who merely thought that it might be. Especially when it is a matter of life and death, is it important that the surgeon should know what he is about, even at the risk of producing metastasis. I have known three instances in which a leg was amputated for what afterward proved to be a syphilitic process. Nevertheless, I am not an advocate of the indiscriminate and unlimited extirpation of fragments from all tumors, and I should be glad to hear the opinions of other members of the Association, particularly as to the advisability of cauterization after diagnostic excision.

Dr. B. F. Schreiner (Buffalo): During the past three or four years I have had approximately five or six hundred cases of cancer referred for diagnosis and treatment. I invariably excise a piece of the tumor, but never follow this procedure by cauterization of any kind. I have never seen any harm follow, so far as I can judge, though it is almost a routine procedure in our dispensary to remove a piece for diagnosis if there be any question as to the exact diagnosis.

Dr. Wood: We have a surgeon with us, and should like to have Dr. Semken discuss the subject from the surgical point of view.

Dr. George H. Semken (New York): I appreciate very much the opportunity to enter into this discussion, though I feel that I must adopt an attitude opposite to that taken by Dr. Ewing. In the first place, incision is supposed to increase malignancy or to produce metastasis, the former by reason of pressure or other mishandling, the latter by opening the lymph-spaces. Practically, it would not make much difference to the surgeon if malignancy were increased, because not much could occur in ten days. But it would make a difference, if there were cancer cells in the lymph-channels; and it is impossible to carry a knife through a tumor without opening some of the blood- and

lymph-spaces, so that the cancer cell is provided with a much readier access to them than under the ordinary conditions of growth.

I have seen a great deal of harm brought about by diagnostic excision. If a patient had but one, and were then operated on immediately, it would not be so bad; but many patients go from one physician to another, and I have seen those who had had seven or eight diagnostic excisions performed. I have not followed my own cases to their end results, so that they are not strictly comparable with Dr. Bloodgood's material. Nevertheless, I can hardly think that he is justified in his statement that all cases in which biopsy has been practiced end fatally for that reason. I think it would be best to restrict diagnostic excision to those cases in which it is imperative, and to warn the patient of the danger of submitting to more than one.

Dr. Wells: My own position is, I think, similar to that of Dr. Ewing; so long as we are not sure that we are not assisting in the spread of a tumor, we should limit biopsy to uncertain cases. The question of the site involved must also be taken into account; thus I am much more inclined to recommend complete removal of a tumor from the breast, where the operation does not cause serious trouble, without waiting for examination of an excised piece of tumor, than I am in the case of a tumor of the tongue or of the larynx.

9. (*a*) The Effect of Continued Inbreeding on the Cancer Rate in Mice

(*b*) The Cancer Rate in Hybrid Strains

Dr. Leo Loeb (St. Louis) *and A. E. C. Lathrop. Read by Dr. Loeb.*

SUMMARY

(a) In these investigations it was our aim to determine the effect which continued inbreeding exerts on tumor rate and tumor age in the various strains of mice which have been under our observation for a considerable number of years, with a view of analyzing the influence of heredity and of external factors in the origin of tumors.

We found that while in the majority of strains the differences in tumor rate and tumor age remained constant in the successive generations, in others certain variations became noticeable as a result of the continued propagation of these strains. In a few cases an increase in the tumor rate occurred in the later generations, but in the large majority of cases in which a change was observed there was a tendency towards a decrease in the tumor rate which in some instances was very marked.

These changes in the tumor rate concomitant with continued inbreeding seem to depend on the following two factors: (1) As a result of long-continued inbreeding in mice certain characteristics of a strain

may change, the strain becoming less prolific and less vigorous. These changes may be accompanied by a lowering in the tumor rate. This was especially evident in the case of the "No. 8" strain which had been inbred through the largest number of generations and through a considerable number of years. (2) In other cases it could be shown that in the course of continued propagation certain families, which were more resistant to certain diseases or which were naturally more prolific or otherwise favored by accidental conditions began numerically to preponderate in later generations. In some cases such families or substrains differed in their tumor rate from the main strain and thus as a result of selection within a strain differences in the tumor rate appeared in the course of continued inbreeding.

Our further investigations confirmed and still further emphasized our previous conclusion that on the whole in the mice with a higher tumor rate the tumors appear at a relatively earlier age than in those strains in which the tumor rate is lower.

In our experiments continued inbreeding was followed in certain strains by a decrease in prolificacy and vigor and the tumor rate varied correspondingly.

We did not inquire whether or not through selective mating a deteriorating effect of continued inbreeding could have been avoided. Our results in no wise depend on the possibility of inbreeding a strain through many generations without experiencing a weakening effect of the inbreeding.

SUMMARY

(b) 1. In order to test our previous conclusions concerning the tumor rate in hybrid strains, we carried out additional hybridizations for which we selected strains which differed markedly in their tumor rate and which had been followed through a number of generations and found constant in their behavior.

As control experiments, hybridizations between strains or families of a similar (either high, medium, or low) tumor rate served. In these cases the offspring showed a tumor rate similar to that of the parents.

2. In selecting for hybridization various groups of "Cream" mice representing a very low tumor strain, and "English Sable" mice representing a high tumor strain, we obtained in the large majority of cases hybrid strains with a tumor rate intermediate between that of both parent strains. In a few cases it approached somewhat the high tumor strain of the English, and in a few other cases the low tumor rate of the cream.

3. In several hybrids between the high tumor strain "English" and the low tumor strain "Silver," which latter was split off from the English, the high tumor rate of the English prevailed. In a number of cases the mice which served for hybridization were followed throughout their life and found to behave typically as to their tumor rate.

4. If we omit the strains in which both parents had a similar tumor rate, we found, altogether, in 24 hybrids, the higher tumor rate to be dominant. In 17 of these the mother strain dominated and in 7 the father strain. In 19 hybrid strains the tumor rate was intermediate. And in 10 strains the lower tumor rate dominated. In 8 of these the mother strain prevailed and in 2 the father strain. The low tumor rate was therefore dominant in approximately 18 per cent of the strains.

5. There does not therefore seem to be a fixed rule as to dominance in the tumor rate. In a considerable number of cases, and especially in well analyzed cases, the result was intermediate.

6. Altogether, in 25 of our hybrid strains the mother strain, and in 9 the father strain prevailed. In 19 strains the result was intermediate. We conclude that either father or mother strain may dominate and that the tumor rate is not in the strict sense of the term a sex-linked character. But the fact that the mother strain prevailed in a much larger number of our cases than the father strain, and that several times (but not in all cases) in reciprocal crosses the hybrids followed the tumor rate of the mother strain, suggests the possibility that, as far as the hereditary transmission of mammary cancer in mice is concerned, the mother may be more potent than the father, and that perhaps under certain quantitatively varying conditions the mother strain may dominate over the father strain. This statement is merely a tentative conclusion at the present time, and needs further investigation before we can consider it as proved.

7. The results of these investigations confirm our previous conclusion, that in the majority of the crosses which we observed the cancer rate is either intermediate between those of father and mother strain, or that it follows the tumor rate of the parent with the higher rate; and that only in a relatively small number of cases does it follow that of the parent strain with the lower tumor rate. On the whole, the heredity of cancer rate and cancer age follows the blending type of hereditary transmission.

8. While there is a distinct relationship between high tumor rate and early cancer age, our observations make it probable that cancer rate and cancer age are to some extent independent of each other.

9. On the whole the different generations, including F_1 and F_2 of the various hybrid strains, showed a concordant tumor rate and tumor age.

10. If we consider our results as a whole, then, we may conclude that in crossing strains which differ in their tumor rate no rule of dominance applying equally to all cases seems to exist. In a certain number of crosses the results are undoubtedly intermediate. In these cases the tumor rate, and to some extent also the tumor age, behave in a manner similar to characters which differ in father and mother merely in quantity, as e.g., the length of organs. But from such intermediate results all kinds of gradations exist, leading on one side to dominance of the strain with the higher, and on the other side to dominance of the strain with the lower tumor rate. However, in our experiments,

dominance of the strains with the higher tumor rate happened greatly to predominate over the opposite extreme.

11. Our results on the whole are therefore in certain respects comparable to the inheritance of sex which R. Goldschmidt studied in hybrid strains of the gypsy moth. Here, also, all gradations from maleness to femaleness were observed in the offspring. Goldschmidt assumes that in different hybrids there are created different quantities of certain substances which, like enzymes, determine according to their quantity the velocity of chemical reactions and the amount of certain substances produced. These latter determine in the hybrids the quantitative variations in the character which is analyzed. According to Goldschmidt, multiple allelomorphs, which in our experiments seem to determine the heredity of spontaneous cancer, depend upon differences in the quantity of a substance present in the different individuals or varieties. In whatever way we may conceive of the character of multiple allelomorphs, our results make it very probable that multiple factors are involved in the heredity of cancer in mice.

DISCUSSION

Dr. Wells: I am glad that Dr. Loeb has brought up the question of inbreeding, because so many who are not familiar with this sort of work have the impression that inbreeding is the cause of the abundance of the tumors. Miss Slye's observations, however, agree with those of Dr. Loeb in showing that inbreeding itself is in no way responsible. When non-tumor strains are inbred, tumors do not appear because of the inbreeding. And when tumor strains are inbred, the incidence of tumors is, if anything, lower than it would be if they were cross bred, since inbreeding lowers the vitality of the stock.

Dr. Clowes: That observation corresponds with one made by Dr. Beebe in his experiments regarding the influence exerted on transplanted mouse tumors by eliminating carbohydrates from the diet. The animals which received carbohydrate-free diet were emaciated and the tumors grew far less rapidly than in the animals that had received a full normal diet. This appeared to be attributable to lowered vitality, malnutrition, or other conditions unfavorable for growth.

Mr. Marsh: We have in Buffalo a spontaneous mouse tumor strain which has reached the seventh generation. There has apparently been no change whatever in the high incidence of tumors through five generations. The sixth and seventh are below tumor age. This corroborates the experience of Dr. Loeb.

Dr. Loeb: I believe that the constancy of strains during continued propagation is of interest in another direction. Certain investigators, for example Haaland and Stahr, have described a change in the susceptibility of certain strains of mice for a tumor, after they had been

removed to another establishment and fed on a different diet. I have examined their evidence and I am not convinced that they were not dealing with a selection of certain families, rather than with an actual alteration in any one strain of mice. I do not believe that there is any evidence so far for the assumption that a change of diet or of climate can alter the character of a strain. Our own evidence shows that the various strains remain constant, and I believe that any assertion of a change should arouse a suspicion that selection of a certain family or of certain individuals has played a part in bringing about the variation.

10. A Quantitative Study of the Physiological Action of Radium

Dr. Alfred Redfield (Boston):

SUMMARY

A physiological reaction well adapted to quantitative study is provided by the fact that the fertilization membrane of the egg of *Nereis limbata* becomes abnormally thick if the egg has been exposed to radiations from radium prior to fertilization.

The change produced in the egg by radiation is irreversible. The magnitude of the physiological effect is not proportional to the product of intensity and time of radiation, the time factor being relatively more important than the intensity factor. An equation expressing approximately the relation between intensity of radiation, I, and time, t, and their physiological effects, V, may be written: $V = a + b \log I + c \log t$. The constants, a, b, and c, depend on experimental conditions, but b is always less than c.

DISCUSSION

Dr. Wood: One interesting thing about these curves is their similarity to the curves obtained by plotting logarithmic density of photographic plates against times of exposure. There occurs what might be called the time of threshold exposure, which seems to exist in radium, in which you get practically no effect; and then the curve runs up, having a straight portion but ultimately falling off toward the abscissa as over exposure occurs. No doubt there is a certain physical similarity between the action of light and of radium, and I should like to ask if the radium curve also shows a falling off after high exposure.

Dr. Bovie: The general idea that the product of intensity and time is a constant in photochemistry is usually accepted more implicitly than the evidence warrants. There are a few reactions in gaseous systems, which are readily mixed, that follow the law; however, there are only four or five such photo-chemical reactions known. In the reactions of photo-chemistry the shape of the vessel is an important factor. This is a factor that in ordinary chemistry we do not have to contend with, except where the sides of the vessel have a catalytic action.

Dr. Clowes: I should like to ask Dr. Redfield what variation he observed in permeability with relation to the matter of conductivity. We found by determining the time factor on the permeability as measured by conductivity, in physical systems in which colloids are involved, that the curves were analogous to his curves. They were similar logarithmic curves, which ran parallel.

I should also like to know whether he observed any difference in the iso-electric point, if any, of the preparations.

Dr. Redfield: In answer to the question of Dr. Clowes regarding permeability, I would say that I have been unable to determine this point in these eggs. It seems almost obvious, however, that any change as great as the alteration I am measuring here must be accompanied by a parallel variation in the permeability of the membrane; but I have no evidence for that view. Nor have I any evidence concerning the iso-electric point in these eggs.

Dr. Clowes: Is it satisfactorily proved that the swelling of the membrane is a question of permeability? Is it a recognized fact that gelatin necessarily shows permeability?

Dr. Redfield: I cannot answer that question. Like Dr. Wood, I have been impressed by the similarity between this reaction and those of photography. The curves are identical with those illustrating the work of Hurter and Driffield on the effect of exposure of the photographic plate. They give a formula relating intensity and time of exposure to the resulting density of the negative, which is similar to the one which describes the reaction in *Nereis* eggs. In their formula there is a factor, i, which expresses the inertia of the plate; it is a measure of the difficulty of getting the process to start. This factor is represented by a function of the intensity of radiation in my formula. It is my idea that there is some secondary reaction which comes to a point of equilibrium determined by the intensity and which governs the rate of the primary reaction.

Another resemblance that is merely superficial is the fact that this is a process which is irreversible. It does not matter how long you wait with the egg before fertilizing it.

Dr. Wood: What was the result of very high exposures?

Dr. Redfield: The curve becomes very irregular.

Dr. Ewing: Were these effects due to the alpha, beta, or gamma rays?

Dr. Redfield: The radium emanations were contained in glass tubes which excluded the escape of the alpha, but not the beta and gamma rays.

Dr. Ewing: Were there any differences in the appearance of the cytoplasm of the egg?

Dr. Redfield: Not until the eggs were fertilized, when the abnormal membranes appeared. The resulting development was also abnormal.

Dr. Ewing: If Dr. Redfield will permit a practical man to make a suggestion, I would say that this paper strikes right at the central point of photo-therapy. I wonder whether he is familiar with work from the Middlesex Hospital on an identical problem, published in the Proceedings of the Royal Society. Not being a physicist, I was not able to follow the argument.

Dr. Redfield: I am not familiar with it. I notice that Dr. Prime and Dr. Wood present figures for the relative effects of intensity and time which follow this notion.

Dr. Wood: There is another factor, which is of importance in practice; that is, the phase of mitosis in which any cell happens to be. This gives another turn to the whole question of time and intensity. Enormous quantities of radium applied for very short periods of time are not nearly so effective as the same amount of radiation spread out over a long time; for in that longer time many more mitotic cells come under the influence of the rays, and it is the dividing cell that is most sensitive.

Dr. Redfield: It would introduce a variation in the same direction.

Dr. Wood: It suggests the use of long exposures wherever possible, as Professor Duane and I have both observed. The relative lack of success with the x-ray may be due to the short exposure, and the more pronounced effect of radium to a longer exposure. This question, however, has not been experimentally attacked, so far as I know.

Dr. William Duane (Boston): I should like to know whether this swelling of the membrane after the egg has been radiated is not a new phenomenon, quite different from anything else known before. If so, it should be carefully investigated.

Dr. Redfield: The reaction is not entirely new, having been very adequately described by Packard, in the Journal of Experimental Zoology for October, 1915. I have tried to duplicate it with other reagents. All of those which cause the membrane to swell do so whether the egg is fertilized or not. With radium the effect is produced only when radiation is followed or accompanied by fertilization. I do not believe that there is anything very characteristic about the abnormalities obtained from radiated marine eggs. They resemble those produced by treatment with various reagents. Nor do I see any

great significance in the fact that eggs radiated during mitosis less often survive than those radiated between mitoses; for there is abundant evidence that the same kinds of egg are particularly susceptible during mitosis to many reagents (alkalies, chloroform, ether, etc.).

Fertilization is accompanied by an increase in permeability. After fertilization the permeability decreases again, to increase at the time of each cleavage.

11. On the Measurement of x-Ray Dosage

Dr. William Duane:

SUMMARY

Several of the scientific commissions in this country that have been founded for the purpose of attacking the cancer problem from all sides are treating patients. At Harvard we see over six hundred patients a year, and at the Memorial Hospital in New York we see nearly one thousand a year. At least 95 per cent of these patients receive radiation treatments, either x-ray radiation, or radium, or both.

Further, a number of interesting researches have been announced on the effects produced by rays on living tissues. As this work progresses it becomes more and more important to estimate accurately both the quality and the quantity of the radiation employed. In view of these facts it does not seem out of place to present to this Association a brief outline of some of the investigations we have been making on the characteristics of the x-rays, and of the methods employed in estimating their penetration and intensity.

x-rays consist of trains of waves travelling out from the x-ray tube with the velocity of light $c\ \left(= 3 \times 10^{10} \frac{\text{cm.}}{\text{sec.}}\right)$ The number of vibrations made per second is called the frequency, γ, and the distance from one wave to the next is called the wave-length, λ. Since, during one vibration, a wave travels along to the position occupied by the next preceding wave, it follows that during one second a wave will travel γ wave-lengths, or a total distance $\gamma\ \lambda$ cm. The distance travelled in one second, however, is the velocity. Hence we have the fundamental equation of wave motion

$$\lambda\gamma = c \tag{1}$$

The two most important characteristics of wave radiation are the wave-length (or frequency), and the intensity. Either the wave-length or the frequency may be used, as the other can be immediately calculated from equation (1).

By intensity of radiation we mean the amount of energy passing per second through a square centimeter of surface normal to the rays.

When electrons in the x-ray tube hit the target they produce the x-rays. The equation

$$V_e = \tfrac{1}{2}\,mv^2 \tag{2}$$

(V = voltage, m = mass of electron, e = its electric charge and v = its velocity) gives the energy of an electron. The impact against the target transforms only a small fraction of this energy into x-radiation.

In practical applications of x-rays a third characteristic becomes important, namely the co-efficient of absorption. This may be defined as the fraction of the radiation absorbed in the next thin layer of matter, taken either per unit thickness or per gram mass of the layer as convenience may dictate.

It is my purpose to state briefly certain simple relations between the above quantities that we have discovered recently.

An x-ray tube produces two kinds of radiation, the general radiation and the characteristic radiation.

Mr. Hunt and I found that the general radiation contains waves of all frequencies up to a certain maximum given by the energy relation

$$V_e = h\gamma \tag{3}$$

where h is a well known physical constant appearing in the analysis of light. Thus the maximum frequency obtainable appears to be proportional to the voltage applied to the tube, and does not depend upon the characteristics of the x-ray tube.

The curves shown indicate the distribution of the energy of radiation among the various wave-lengths.

The frequencies of the characteristic radiation depend upon the substances composing the target in the x-ray tube, and not upon the voltage or current.

The curves shown illustrate the K series of lines of the chemical element rhodium. Particular interest attaches to the K lines of a chemical element, because they represent the highest frequency vibrations known to be characteristc of that element.

Dr. Webster, working in our laboratory, has shown that in order to produce the K series of lines the voltage must be at least as great as that given by equation (3) in which γ is the highest frequency of the series.

Professor Blake, Mr. Hunt, and I have measured the highest frequencies in the characteristic x-rays for over one-third of the chemical elements and find that approximately they obey the law

$$\gamma = \gamma_o \, (N - 3\tfrac{1}{2})^2 \tag{4}$$

in which γ_o is a constant taken from spectrum analysis, and N is the atomic number of the chemical element.

The atomic number of an element represents its position in the series of elements beginning with hydrogen as one and ending with uranium (the element of largest atomic weight) as 92.

The law connecting the velocity of the electron required to produce the K radiation with the atomic number appears to be exact within the limits of error of the experiments. It may be stated thus

$$N = N_o \, (N - 1\tfrac{1}{2}) \tag{5}$$

in which N_o represents a constant. The curves shown illustrate the laws.

Some years ago I collected all the data I could find bearing on the co-efficient of absorption of metals for x-rays, and worked out the relation between this co-efficient and the wave-length of the ray. The curve shown represents this data.

It appears that the co-efficient of absorption of an element increases as the cube of the wave-length, and may be expressed by the equation

$$N = k\lambda^3 \qquad (6)$$

except near the characteristic lines of that element. For the mass co-efficient of absorption of aluminum $K = 14.7$.

Some time ago I designed an instrument for measuring the intensity and wave-lengths of x-rays. The drawings shown illustrate this instrument.

The x-rays pass through a small metal box and the ionization current through the air contained in this metal box is measured by means of a galvanometer. The reading of the instrument gives the intensity and the ratio of the two readings; a_1 with and a_2 without a thin sheet of aluminum (thickness d) gives the linear co-efficient of absorption from the formula $\mu = \frac{1}{d} \log \frac{a_2}{a_1}$. From this, by means of equation (6) the effective wave-length may be calculated.

By effective wave-length I mean a kind of average wave-length that has the same co-efficient of absorption as the whole x-ray beam.

DISCUSSION

Dr. Wood: I should like to have Dr. Duane tell us more about the calibration of the box.

Dr. Duane: It is a simple resistance box. With a given volume of air in it, one can calculate the intensity of the ray and the effective wave-length.

Dr. Redfield: I should like to ask Dr. Duane whether a similar method could not be developed for determining the quality of the tubes in the use of radium.

Dr. Duane: The only trouble is that the ionization current is very small, so small that one cannot measure it by the galvanometer. Other means are therefore necessary. The simplicity of this method consists in the use of the galvanometer, taking the reading on the scale. If one were to measure with some method involving the use of a stop watch, etc., the process would be more complicated, although the principle would be the same.

12. Recent Advances in Our Knowledge of the Physiological Effects of Radiation

Dr. W. T. Bovie:

SUMMARY

Various theories have, from time to time, been advanced to explain the physiological action of radiation. These theories, since they are attempts to correlate observations with the aggregate of known facts, are but reflections of the philosophy of their times. The writer suggested in 1913, in discussing some experiments upon the temperature coefficient of the photo-coagulation of egg albumen, that the observed effects might be correlated by supposing that the radiation causes chemical changes in the more complex and unstable components of the protoplasm and that the subsequent physiological disturbances are the results of these changes. Undoubtedly, many students of the subject have entertained similar views. Nevertheless, in discussing the subject with investigators and clinicians it has often appeared to me that the ideas were not so clearly crystallized in their minds as in mine. The theory has been useful in directing my investigations and in interpreting my results, as well as the results of others. This paper is a brief statement of some of the results of a number of experiments calculated to throw light upon the nature of these photo-chemical changes and upon their relation to the resulting physiological disturbances.

It was pointed out in the experiments referred to above, that egg albumen becomes sensitized to heat when exposed to ultraviolet rays and coagulates at temperatures much below the "coagulation temperature" of the unexposed albumen. I have found that the protoplasm of *Paramecium* becomes sensitized to heat when exposed to ultraviolet (fluorite) rays and cytolizes at temperatures which are not above the optimum temperature for growth and reproduction of the unexposed organisms. The protoplasm of the fronds of *Laminaria Agardhii* is sensitized to heat by exposure to the rays from radium emanation, so that its resistance to the passage of an electrical current is very greatly decreased by brief exposures to temperatures which do not affect the non-radiated controls. It appears that heat sensitiveness will be found to be a frequent peculiarity of radiated protoplasm.

I have found that it is possible, by selecting ultraviolet radiation of proper wave-lengths, to localize the place of action of these rays within the cell. The localization is due to the fact that extreme ultra-violet (fluorite) rays are largely, if not quite entirely, absorbed in the cytoplasm of paramecia, while the longer ultraviolet (quartz) rays are transmitted through the cytoplasm and are absorbed in the nucleus.

When a paramecium is exposed to fluorite rays (wave length in the neighborhood of 1600 Ångström units) for a sufficient length of time, it cytolizes. Sub-cytolytic exposures may be caused to give rise to cytolysis by a slight elevation of the temperature of the culture drop in which the organism is living (heat sensitization).

In one hour a paramecium so completely recovers from a brief exposure to fluorite rays that the effects of a second brief exposure are no longer reinforced by the remaining effects of the first.

Cell division is not inhibited by fluorite rays, however long the exposure. When a paramecium is exposed to quartz rays (wave length 2750 Ångström units) for a sufficient length of time it cytolizes. Sub-cytolytic exposures to quartz rays inhibit cell division. The duration of the inhibition increases with increase in the length of exposure. Short exposures cause brief periods of inhibition. Brief periods of inhibition are followed by acceleration, so that the number of descendants of the radiated paramecium may exceed that of the non-radiated control. With longer exposures, the inhibition may continue for several days. After long periods of inhibition the organisms do not recover, but become transparent and finally die.

The rays from radium also inhibit cell division in paramecia. In one experiment cell division was inhibited for seventeen days, after which division again occurred. In the meantime the control, dividing once every twenty-four hours, had potentially produced over one hundred and thirty-one thousand individuals. During this period of inhibition the radiated organism became thin and transparent and lost its power of locomotion.

The results of this experiment are interpreted as indicating that the physiological effects of the radiation are the results of photo-chemical changes taking place in the protoplasm at the place where the rays are absorbed.

DISCUSSION

Dr. Clowes: I am interested in Dr. Bovie's curves with sodium and calcium because I am going to exhibit somewhat similar curves obtained with purely physical systems containing no proteins, but consisting simply of oil, water, soap, and filter paper, in which, consequently, the effects observed cannot be attributable to any action on proteins.

Dr. Bovie's results suggest colloidal effects. For example, it is well known that the aggregation of a colloidal suspension may be effected far more readily by adding a given amount of precipitant at one time than by adding the same amount divided in several instalments. The irregularity in curves followed by subsequent reversion to the normal observed by Dr. Bovie is something with which we are already familiar in experiments both upon living cells and physical systems under the influence of electrolytes. The close resemblance between the proportions of certain electrolytes in sea-water, in the blood of mammals, and in other environing media of protoplasm, suggests that protoplasm may represent an adaptation of certain colloidal constituents to their original environing medium. The difficulty of getting results with physical systems which perfectly parallel those obtained with living cells may be attributable in a measure to the extraordinarily fine

balance attained by protoplasm during the long period of time occupied in the process of evolution. An emulsion prepared ten years ago exhibits superior properties to one recently prepared, and when it is contained within the meshes of filter paper, jelly, or other carrying medium, the curves exhibited are much more perfect than when a crude emulsion is employed.

Dr. Bovie: As for the physical changes involved in cytoplasm I may say that while studying the photo-cytolysis of paramecia by fluorite rays I endeavored to find a physical explanation of the phenomenon. The visible changes had such a strong resemblance to the breaking down of emulsions that I decided to investigate the effects of rays upon emulsions. I discussed the matter with physicists, and was assured that the experiments would give negative results because it was impossible for radiation to affect surface tension sufficiently to affect the equilibrium of an emulsion. I had, however, observed that small drops of water, paraffin oil, carbon tetrachloride, or in fact, of any of the substances that I tried, could be made to creep about by radiating them with fluorite rays. I made up emulsions of olive oil and dilute alkali and found that they broke down very quickly when radiated with fluorite rays. It was possible actually to bore a hole through a stiff "mayonnaise-like" emulsion made by beating olive oil and dilute alkali. I have not found an adequate explanation of the breaking down of these emulsions, and have never published the experiments. I am not supposed to be able to explain the mechanism of the photo-cytolysis of paramecia, but it does not redound to my credit that I am unable to explain so simple a thing as the breaking down of an emulsion of soap and water, by fluorite rays.

Dr. Wood: I feel that we are all rewarded by this discussion, which has been very interesting to me as evidence that we are coming closer to some possible explanation of what is going on inside the cell. Dr. Bovie's work on the paramecium and its nucleus interests me particularly because I have long held the belief that too small doses of radium stimulate the tumor cell, while larger ones may check its growth. Tumors, however, are such fluctuating material that it is difficult to advance an absolute proof.

Dr. Bovie: I am unable to offer an explanation of the stimulating effects of the rays. I have tried to obtain a stimulation without an inhibition but so far have not succeeded. A stimulation following an inhibition strongly suggests the blocking of a series of reactions, the stimulation occurring when the blocking breaks away.

13. Recent Changes in the Mortality from Cancer

Dr. Frederick L. Hoffman (Newark):

SUMMARY

In my treatise on "The Mortality from Cancer Throughout the World," the final conclusion was advanced, and without hesitation, that "the evidence of cancer increase throughout the world is an incontrovertible statistical fact and absolutely conclusive." During the intervening three or four years a considerable amount of new information has been forthcoming but no evidence has been produced to necessitate a modification of the conclusion that the observed increase in the cancer death rate throughout the world is real and not apparent or in consequence of improved diagnoses, changes in the methods of death certification, etc.

The human death rate is such a composite factor of human existence that it would be hopeless to contend for universal laws or principles governing specific disease occurrence proportionate to the populations affected, with a due regard to age, sex, and racial distribution. Extended statistical analysis never fails to bring to light new contradictions in cancer mortality experience but in the main the conclusion would seem entirely sound, and in strict conformity to the facts, that "cancer is much more common than has generally been assumed to be the case," and that "the mortality from the disease throughout the civilized world exceeds 500,000 deaths per annum, and is in the United States about 80,000 at the present time." Efforts have been made from time to time to contradict the conclusion that the increase in the mortality from cancer is real, and not apparent, but the evidence brought forward can not be considered satisfactory for the purpose, more so in view of the fact that the proof has been statistical rather than medical or surgical, indirect rather than direct, or general rather than specific.

In a recent contribution to The Journal of Cancer Research, Prof. W. F. Willcox, of Cornell University, has revived the controversy by a pressentation of new data for the city of Frankfort, in continuation of the earlier analysis by Newsholme and King. The argument is entirely statistical and, broadly speaking, without real medical or scientific value in the vast field of cancer research. The data as such, which are quite extensive in numbers and point of time, were not subjected to critical medical consideration by one qualified to do so, and there is nothing to indicate that each and every death certificate was gone over with the necessary care. In fact, very little is made of the new Frankfort data by Professor Willcox, and most of his discussion has reference rather to general questions of mortality analysis—that is, variations in the death rate on account of variable factors such as age and sex distribution, etc. The misleading classification adopted by Newsholme and King is adhered to regardless of the obvious urgency

of a more precise and scientific differentiation of cancer recording as to accessibility or inaccessibility, for purposes of initial or terminal diagnosis. The cause of cancer research is hindered rather than advanced by mere controversial discussions of this kind, which, at least to the general student of the subject, must make confusion worse confounded.

It is not my intention to review the article by Professor Willcox in all its essential details at the present time, nor would such a discussion serve a very practical purpose where so much really useful work requires to be done. The article bears intrinsic evidence of an essay in statistical controversy rather than an attempt to further important purposes in connection with cancer research. Since one of the main conclusions implied is that the apparent increase in cancer is probably chiefly in consequence of improved diagnosis, it need only be said that no specific evidence whatever is advanced that cancer diagnosis has been seriously at fault in any special branch of cancer mortality statistics; for, after all, real progress in cancer mortality analysis can only lie in the direction of a thoroughly critical and qualified consideration of carefully selected groups of cancer facts, such as cancer of the breast, the buccal cavity, the uterus, etc.

Professor Willcox prefers the dual classification of accessible and inaccessible cancers adopted by Newsholme and King on the basis of an obviously faulty classification, to the strictly scientific method suggested by Dr. Bashford, of the Imperial Cancer Research Fund, including an intermediate group of borderline cases clearly belonging to neither one nor the other. Although preferring the dual classification to the triple classification, Professor Willcox, in his own data, includes a group of cases with the position undefined, which is sufficiently large to invalidate practically all of his conclusions, at least for the earlier years. For illustration, during the years 1860–1866, out of 1603 cancer deaths in the city of Frankfort, 359, or 22.4 per cent, represented deaths in which the position was undefined. Although "for the sake of simplicity" Professor Willcox gave the preference to the dual classification, he certainly was not justified in referring to the deaths assigned to the position "undefined" as "*insignificant*." Furthermore, his admission that arbitrary assignments in the dual classication as followed were unavoidable overlooks the fact that certain cancers, such as those of the skin, other parts of the mouth than the tongue, and other external parts of the body, which should have been classed as accessible, were not so classified because they were not distinguished in the printed sources as used by Newsholme and King, is certainly not a scientific argument. To draw false inferences because of inherently faulty data does not advance the cause of scientific research.

The use of the Frankfort data in connection with cancer mortality investigations is one of the curiosities in medicine. Why so much pains should have been taken with an analysis of statistics practically inaccessible for verification, and made in a city subject to entirely different methods of registration practice, death certification, etc.,

rather than any one of the larger cities of this country, is inexplicable. The tabulation of the original data as, fortunately, included in an appendix to the article by Professor Willcox, clearly shows that the information is thoroughly self-contradictory throughout the whole long periods for which the data have been brought together. No such classification as is followed by the city of Frankfort is in current use in this country or in any other country which has adopted the international classification. The number of cancers with the position undefined is 688, out of 9242, but there are other cancers specifically defined, such as those of the nervous system, the bones, etc., which do not admit of a differentiation as to whether accessible or inaccessible, as the case may be. If, therefore, any real value is to be attached to the Frankfort data they should be entirely rearranged and a complete tabular presentation should be made according to the organ or part of the body affected. As thoroughly made clear in my discussion of methods of classification, etc., consistent uniformity is of the first importance.

Qualified observations on the increase in cancer are practically co-extensive with the progress of medicine, at least during the last one hundred and fifty years. It would be truly inconceivable that, say four or five generations ago, cancers of all forms, organs, and parts should have been as common as at the present time without the fact having sufficiently attracted the attention of the medical practitioners of the period. Superficial conclusions regarding erroneous cancer diagnosis almost invariably overlook the fact that the diagnosis is much less difficult *at death* than is generally assumed to be the case, however difficult the initial diagnosis of cancer may be in the case of any particular organ or part. This conclusion applies in a large measure to internal cancers as well as those of the external, or readily accessible internal, organs or parts. So careful an observer as Christopher Turner Johnson, in a practical essay on cancer to which was awarded the annual prize for 1808, by the Royal College of Surgeons, and of which an American edition was printed at Philadelphia in 1811, described only cancer of the breast, the uterus, the generative organs, the skin, the eye, and the tongue; yet even Johnson, who dedicated his essay to Astley Cooper, the most eminent surgeon of his time, gives expression to the opinion, based on an examination of the Bills of Mortality, that "the proportional number of deaths from cancer in a given time has of late years been on the increase." This increase has been so very considerable and so persistent from year to year that it would be truly inconceivable that material medical or statistical errors should invalidate the conclusion that cancer at the present time, in proportion to population, is relatively twice as common as it was forty years ago.

Arguments that cancer is not on the increase may conversely be construed as conclusive proof that cancer is on the decline, for no death rate from any cause ever remains stationary, but continuously fluctuates, with generally either a pronounced upward or downward

tendency. The cancer death rate, even for recent years, has not always shown an increase, and it is extremely significant that during the year 1917, for illustration, the cancer death rate of 35 American cities should for the first time in eleven years show a distinct downward tendency. As shown by the table following, the death rate has almost continuously increased from 77.8 in 1907 to 93.9 in 1916, per 100,000 of population, but it declined to 92.8 per 100,000 during the year 1917, regardless of an enormously enhanced public interest in the cancer question, both on the part of the medical profession and the laity. If, therefore, the argument so frequently advanced, that the increase in cancer is in consequence of improved diagnosis, then it is a fair assumption that the same authorities would hold that the mortality rate from cancer had decreased, for the opposite reasons.

Mortality from cancer in thirty-five American cities, 1907–1917

YEARS	POPULATION	DEATHS	RATE PER 100,000 OF POPULATION
1907	15,491,267	12,045	77.8
1908	16,014,235	12,302	77.4
1909	16,464,980	13,413	81.5
1910	17,012,722	14,206	83.5
1911	17,442,980	14,562	83.5
1907–11	82,426,184	66,618	80.8
1912	17,895,192	15,367	85.9
1913	18,322,037	16,393	89.5
1914	18,756,577	16,955	90.4
1915	19,209,832	17,306	90.1
1916	19,636,951	18,439	93.9
1912–16	93,820,589	84,460	90.0
1917	20,113,139	18,657	92.8

The details of this analysis, by single cities, are not available at the present time further than that, comparing the 1917 rate with the average rate for 1912–16, the rate increased in 24 out of the 35 cities, the remaining 11 cities in which the rate decreased having been Buffalo, Dayton, Hartford, Indianapolis, Milwaukee, Newark (N. J.), New Orleans, Paterson (N. J.), Richmond, Seattle, and Worcester. The favorable changes in the death rate during 1917, however, are much more pronounced when the record for the year is compared with the corresponding rates for 1916. It therefore admits of no controversy that exceptions to the general principle of cancer increase may be met with for short periods of time, although the general trend of the rate for the large majority of representative localities may continue in an upward direction.

It admits of no argument, of course, that the death rate from any specific cause must at some time reach an approximate maximum point

of relative frequency. Considering the fact that a perceptible number of deaths from cancer are annually prevented by surgical operations, or otherwise, and in all probability to a much larger extent at the present time than in former years, the observed increase in the cancer death rate is so much more significant. Considering further that in certain countries like Switzerland the cancer death rate exceeds 100 per 100,000 of population, or 1 per cent, whereas in this country, for the registration area, the rate has not yet reached 80 per 100,000, and for the 35 cities only 90 per 100,000 during the five years ending with 1916, it is self-evident that we are approaching a point of maximum frequency for the country at large which may be conservatively placed at 120 per 100,000 of population, although rates have been reported in excess of that amount for a number of thoroughly representative countries and localities. The European war will necessarily impair all future cancer statistics, and a correction for profound alterations in the age and sex constitution of the populations concerned will become most difficult, if not statistically impossible. Even in the case of our own country, allowance for population changes will have to be made in the future in consequence of our participation in the European war, although, of course, to a much lesser extent than, for illustration, in the case of Germany, Austria, Belgium, and France.

Without further enlarging upon the question, I can not but feel that the evidence of a material increase in the cancer death rate during the last generation is sustained by such an overwhelming mass of statistical evidence that statistical arguments to the contrary belong to the realm of hopeless conjecture and idle speculation.

DISCUSSION

Dr. James Ewing: I think it is remarkable that two such distinguished and unquestionably accomplished statisticians as Dr. Hoffman and Dr. Willcox, dealing with a clean-cut question and having at their disposal much the same data, should come to conclusions which are not in unison, and which, in fact, are in some respects diametrically opposed. There must be some clear and definite reason for this difference. I do not pretend to know just what it is, but I have an idea that Dr. Hoffman hit the nail on the head in his last sentence, when he attributed it to the incompetent work of the physicians on whom a part of these statistics depend. I do not want to destroy Dr. Hoffman's confidence in the diagnostic ability of his physician, or of physicians in general; I do not want to underestimate the capacity of the medical profession to diagnose; but I am quite sure that if Dr. Hoffman were as familiar as we pathologists are with their inaccuracy, even under the best circumstances, I do not think he would be quite so severe on Dr. Willcox's conclusions. We are only too well acquainted with the difficulty of getting reliable information from any considerable proportion of men even of average medical intelligence. But I do not think that Dr. Hoffman understands the great complexity of the

disease that we include under the term cancer, or that he is familiar with the medical side of the problem. While I am in no sense an apologist for Dr. Willcox I strongly advise all who have not read his article to do so. I was more or less responsible for the general color of Dr. Willcox's views, and spent a considerable amount of time in explaining to him the sources of error in his statistical data. I think it possible to develop that subject even more than he has done, yet just as the matter stands now he assumes that over one-half of the recorded increase can be explained on this basis. In fact, Dr. Willcox goes so far as to say that the remainder of the increase may possibly be susceptible of similar explanation.

As a matter of fact, the subject of the increase of cancer is, in my opinion, not ripe for purely statistical study. In order to reach proper conclusions, it is necessary to study the psychology of the doctor, the psychology of the public, and a great many other complex factors. This is necessary, in order to determine whether a patient actually did die of cancer when the death is recorded as such.

Finally, as to the deplorable expense mentioned by Dr. Hoffman, we spent only one hundred and fifty dollars in this investigation, for which I was glad to be able to secure the funds.

Dr. R. A. Lambert (New York): Dr. Ewing has expressed my own ideas much better than I could have presented them. It seemed to me, as Dr. Hoffman was speaking of the crudity of the data, that if any sum of money was to be expended it should go toward improving the data, and not toward investigating the crude data that we now have.

There are limitless possibilities for improvement of the statistics. Thus data are to be obtained that are not open to the sources of error emphasized by Dr. Ewing. These are autopsy data, which are probably included in the material that Dr. Hoffman has analyzed; I am not sure that they are kept separate. The New York Board of Health follows the excellent plan of sending blanks to the hospitals, which the pathologist fills out after having completed his histological examination. I do not know whether the data thus obtained are kept separate from the other statistics, in which the source of error is great, but it should be. It seems to me that a city like New York could accumulate in ten or fifteen years statistics that would be extremely valuable, and the plan might be followed in other cities to great advantage. Again, a law might be passed providing for an autopsy in the case of every person who dies of anything that might be cancer, though I suppose this is too much to hope for in this country.

Dr. Woglom: I have no preconceived notions on the question whether the number of deaths from cancer is increasing or not, and ask the following question merely for my own information. It has been suggested that if the rising curve representing a constant increase in the cancer death rate were to be produced backward, it would, sooner or

later, reach zero; in other words, at some time in the past there may have been no deaths from cancer. I wish to known whether a statistician would regard this as a reasonable criticism of the view that the number of deaths from cancer is increasing.

Dr. Wood: An interesting example of the reaction between the physician and the diagnosis is now being observed in England. The number of deaths ascribed to cancer is increased over the records of the most recent preceding years, but the deaths from sarcoma are fewer. In the report of the Registrar General it is pointed out that the decline in sarcoma mortality is probably associated with the increase in that from undefined cancer, for the former had been increasing and the latter decreasing for many years up to the commencement of the war, since which time the movement seems to have been reversed. The most probable explanation, according to the Registrar General, may be found in the withdrawal from civil practice of so many of the younger physicians, who would probably distinguish sarcoma as such in their certificates somewhat more frequently than the older men, upon whom the added burden of their work has now fallen. If we did not know what is going on in England we should be at a loss to understand the reversal of the figures; and this is a concrete illustration of how our statistics vary under different methods of investigation.

Dr. Hoffman: In reply to the statement by Dr. Ewing that perhaps the question of an increased cancer death rate cannot (if I quote him correctly) be properly dealt with statistically, I would say that I might be inclined to agree with him if the analysis is to be made by men who have no medical knowledge and understand only statistics. Nothing is more misleading than mere statistics, and most of the analogies fail precisely on that point.

A very interesting question was raised by one of the speakers, who inquired as to the justice of producing the curve backward. I think that this would be a fair procedure, and that the fact that it would reach zero indicates that cancer is a disease of modern life. There are many competent observers who insist that cancer is rare among primitive people, and who are therefore certain that cancer is a modern disease.

Again there is reason to believe that the great range of frequency as regards the different parts of the body affected, gastric cancer being especially common, is a modern phenomenon due to completely new modes of life. Surely, if those who have practiced for a generation among Indian tribes have not been able to find any evidence of the occurrence of cancer in them, or to discover any evidence of death from malignant disease of the bones by examining their remains, it is a reasonable assumption that the disease must be rarer in this race than in others. Now I am by no means opposed to the idea that in primitive people of past generations cancer has occurred; but it must have been very rarely.

When we examine the records of the past, we find that the increase of cancer has long been a subject of discussion. Thus, in a book written by a prominent English physician one hundred years ago, and dedicated to Sir Astley Cooper, himself the author of a book on cancer of the breast, the following sentence occurs: "It appears that the number of deaths from cancer in a given time has, of late, been on the increase." In the older records of Vienna, cancer was only mentioned once in four or five thousand deaths; and the literature of two hundred and fifty years ago shows that while cancer was recognized, it was referred to much more rarely than at the present time.

I would not oppose the idea that there may have been a time when cancer was actually at the zero point. Its modern increase I believe to be due, as I have already said, to the extremely complex conditions of modern life. The responsible factor may be an error in metabolism, an excess or deficiency of some mineral salts in the diet, or the ingestion of some irritating substance; or there may be a multitude of causes. There is no reason at all, in my opinion, to oppose the idea that cancer has increased; indeed the wonder is that it has not increased much more, when we contrast modern habits of eating with those that prevailed even earlier in my own lifetime, forty or fifty years ago.

I am well aware that this is by no means an answer to the question of the increase of cancer; but it is an indication that these factors have not been overlooked by those who are committed to the view that cancer is actually on the increase.

Something was said about autopsies. It may interest the Association to know that all the autopsy records of the Johns Hopkins Hospital are in my office and have passed through my hands. They are extraordinarily complete as to detail, and I am sure that no German or Austrian hospital can equal them in this respect. All have the microscopic findings attached. In other words, so far as any reasonable statement of facts can go, I believe that the Johns Hopkins data are practically conclusive; and while their analysis has not yet been completed, it has gone far enough to discount the alarming statements of those who would indict the whole medical profession after discovering a few cases of undiagnosed cancer.

I realize fully that one who is not an experienced physician; and, more than that, an experienced surgeon; and still more than that, an experienced pathologist, microscopist, and biologist, as one should be in addition to his qualifications to deal with statistics, is greatly handicapped; and I have therefore been reluctant to commit myself too deeply, knowing my lack of medical knowledge. But there is not a leading surgeon from Maine to California with whom I have not discussed this question; I have seen one hundred operations for cancer in the Mayo Clinic alone; I have examined the records of the University of Minnesota, and I am satisfied that we shall never get anywhere by a mere statistical analysis and re-analysis of foreign data. The only hope of progressing anywhere lies in a thorough examination of the facts under our own control, within our own experience, in our own country. Even though we were limited to a few thousand cases, we should then advance, instead of being led into useless controversy.

14. (*a*) Preliminary Note on the Action Exerted by Antagonistic Electrolytes on the Electrical Resistance of Emulsion Membranes

Dr. G. H. A. Clowes:

SUMMARY

Previous determinations have shown a high content of K, Na, and soap, and a low content of Ca, in rapidly growing tumors; and since the permeability of normal tissues is known to be increased by salts of Na and K, and decreased by those of Ca, it appeared desirable to determine the influence exerted by the electrolytes in question on the conductivity of purely physical emulsion systems of the type previously described by the writer. When solutions of NaCl and $CaCl_2$ having an equal conductivity were allowed to act on emulsions contained between filter paper diaphragms, NaCl increased and $CaCl_2$ diminished the conductivity; and it was found possible by means of alternating treatments with the salts in question to produce rhythmical variations in conductivity corresponding exactly with those observed by Osterhout in his experiments with marine organisms.

That the electrical conductivity affords an accurate index of the permeability was proved by passing the solutions in question through emulsion systems, when it was found that NaCl greatly increased the speed of flow and $CaCl_2$ gave a result approximately the same as that given by distilled water or sea water.

These results, together with those recently published by Hirschfelder, correspond with the writer's experiments on antagonistic electrolytes carried out by means of the drop system, and support the contention previously advanced that variations in permeability of the protoplasmic membranes are attributable to the action of electrolytes and metabolic products on a delicately balanced emulsion system, and that proteins simply afford a supporting structure for the mechanism in question. This view is supported by the fact that blood plasma exhibits only a slight variation in conductivity, either on clotting or on subsequent treatment with antagonistic electrolytes.

The fact that emulsions, when admixed with $CaCl_2$ without a filter paper diaphragm, give only variations in conductivity as compared with those carried out with a diaphragm, indicates that the contact mechanism lies in the emulsion in the pores of the filter paper diaphragm.

14 (*b*) Preliminary Note on Electrical Resistance of Tumor Tissues

Dr. Clowes:

SUMMARY

Preliminary determinations of electrical resistance indicate that cancerous tissues are more permeable than normal tissues, and that the permeability appears to be directly proportional to the speed of

growth. Similar determinations on plant galls supplied by Dr. Erwin F. Smith, indicate that plant tumors are more permeable than normal plant tissues. This method might afford a means of determining the relative malignancy of tumors.

DISCUSSION

Dr. Bovie: I should like to call Dr. Clowes' attention to the fact that Dr. Brooks has recently published some experiments in which he used sections of laminaria instead of filter paper, and obtained results similar to those reported by Dr. Clowes. Sodium chloride passes through very quickly, while balanced salt solutions pass through much more slowly or not at all.

Dr. Redfield: The curve shown by Dr. Clowes, which represents first a decrease and then an increase in permeability, is approximately the form of the differential of the equation expressing the integral of the effect of radium on the egg of *Nereis;* and this fact seems to suggest some fundamental underlying principle in both cases.

There is a question I should like to ask Dr. Clowes concerning the work of Zwaardemaker, which has recently appeared in the American Journal of Physiology. He found that he could replace the potassium in Ringer's solution by a number of radio-active substances, provided the quantity was sufficient to be just as radio-active as the potassium omitted, and still maintain a balanced physiological solution. He concluded from this that the physiological action of potasssium was due to its radio-activity. I wonder whether that was a justifiable conclusion, or whether it would be better to think of potassium and of radiations as both increasing permeability, and so being antagonistic to other ions which decrease permeability.

Dr. Duane: I should like to ask Dr. Clowes whether or not he has used iridium salts. They are much more radio-active than potassium.

Dr. Clowes: The conductivity results with the filter paper systems have, in our experience, proved more reliable than those obtained with living tissues. The results on conductivity with plant tissues are far more satisfactory than with animal tissues; nevertheless, the method may be suggested as one which might be utilized by pathologists to determine the relative speed of growth and the malignancy of a given tumor. The fact that embryonic tissues exhibit greatly increased conductivity, and that marine organisms subjected to toxic influences, unfavorable chemical environment, sewage, etc., likewise exhibit an increased conductivity, suggests the possibility that investigations on these lines might throw some light on the cancer problem.

Without introducing any further mathematical discussion, it may be stated that the physical systems described above, reacting as they do

with calcium, magnesium, sodium, potassium, etc., appear to parallel living tissues closely and should afford some information regarding protoplasmic structure.

I have not used iridium on the systems just described. There is much in radio-activity that suggests the advisability of investigating the effects produced in their relation to the action of adsorbed hydroxyl ions in colloidal systems.

As regards the antagonistic effects exerted by sodium and potassium, on the one hand, and calcium and magnesium on the other, it should be noted that, in surface tension experiments with capillary pipettes and soap films, under approximately neutral conditions sodium and potassium antagonize calcium and magnesium; with increasing alkalinity magnesium appears to function, like sodium and potassium, as an antagonist to calcium; and with increasing acidity potassium tends to antagonize sodium. It is therefore very important to consider the adsorption of hydrogen ions and hydroxyl ions in such colloidal systems.

Dr. Bovie's experiments with potassium remind me of certain experiments which we made some time ago with soap jellies containing varying proportions of sodium and potassium. When such jellies contain only sodium they are solid at the normal temperature of sea-water, but the addition of a small amount of potassium equal to that occurring in marine systems produces a semi-fluid jelly at the temperature in question. The extent to which the melting point of such a jelly may be raised by the addition of a small amount of potassium is really remarkable.

The influence of temperature on Dr. Bovie's curves would certainly appear to indicate that he is not dealing with an ordinary chemical reaction in a homogeneous system, but with a colloidal system in which adsorption and surface tension effects come into play. While chemical reactions exhibit an increase in velocity with rising temperature, surface tension diminishes in a manner corresponding with Dr. Bovie's curves.

Dr. Ewing: These physicists remind me of the advice that, "Even in philosophy, it is necessary to use terms with meaning." I have no doubt that the terms are full of meaning to them, and that they refer to definite facts in physics; but I have listened to similar discussions in this society for several years, yet have listened in vain for any attempt to translate these terms for the pathologist. Is it not possible for the physicist to explain pathological changes from the view point of the physician, and to interpret phenomena from this standpoint, thus transforming the discussion from one largely meaningless to the pathologist into one that is full of significance? If this can be done, you may be sure of introducing some important changes into pathological interpretation, for the physicist can explain many of these things better than the pathologist can.

Dr. Bovie: I want to ask Professor Ewing whether the material that I have attempted to present this afternoon was unintelligible; for I really tried to eliminate all the terms that I thought would not be understood. Handicapped as I was this morning by pathological terms, I can fully realize the difficulties that confront one who is listening to a paper outside one's own field, even when the speaker strives to employ only such terms as belong to the ordinary scientific category. However, I challenge Dr. Ewing to prove that it was the mathematics that was not intelligible.

Dr. Ewing: Not from the physical standpoint; but the point that I was trying to make is that one can neither see these things going on in the living cell nor interpret the morphology in the terms that have been employed here.

Dr. Clowes: We have tried to investigate questions of surface tension in the amoeba, for example, and also to interpret, in terms of actual physical equilibrium of a two or three phase system, the phenomena of karyokinesis. But until one has one's self some sort of definite idea of what one is driving at, it is not easy to employ terms that shall be intelligible to another.

I think that what I did not make clear to the pathologists is this: A particle that is suspended in water—oil, say—may be dispersed by an agent that lowers the surface tension. When we use soap, we disintegrate the particles of oil and spread them out. A little salt will further disperse the oil and lower the surface tension, and give more and more of these particles; because the negative chlorine ion is more active than the positive sodium ion.

I am going to send Professor Ewing some articles that I have written, and ask him whether they are unintelligible. If so, I shall start all over again. I want to put before pathologists the material that we have worked on, for I realize that I myself cannot apply it, and that I must leave the application to men like Dr. Ewing.

15. The Relation Between Spirochetes and Mouse Tumors

Dr. Theodor Mueller (Buffalo):

In 1905 Borrel found spirochetes in nonulcerated mouse tumors, but apparently did not attribute much significance to them. Wenyon, and Breindl and Kinghorn, in 1906, found spirochetes in the blood of non-tumor mice which had been experimentally infected with trypanosomes, and had no difficulty in transmitting the spirochetes subcutaneously and intraperitoneally to other mice. In 1907, both Gaylord and Calkins described spirochetes in mouse tumors. Gaylord saw them in sixteen secondary tumors (adenocarcinoma) from three different strains. They were most numerous at the margin and in the connective tissue of the tumors, only a few being discoverable in the

center. These authors succeeded in finding spirochetes in ten primary tumors also, though never in any tissue of healthy mice, and therefore suggested that these organisms probably bore some etiological relation to the genesis of mouse carcinoma. Soon afterward Tyzzer described spirochetes in four mice, one of which had been successfully inoculated with tumor, while the other three were immune. Deetjen, in 1908, saw spirochetes in two different strains of tumor mice, and also in the subcutaneous tissues of mice which were immune against tumor inoculation. But he could not find spirochetes in healthy mice or in mice with primary tumors; and as he observed the development of a secondary tumor without spirochetes, he reached the conclusion that in all probability the spirochetes had nothing to do with the etiology of tumors.

The present experiments were undertaken with the object of definitely clearing up the relation between spirochetes and mouse carcinoma. In these experiments, only mice from the laboratory of the State Institute for the Study of Malignant Disease were employed. All transplantations of tumor were made with a trocar, which was introduced into the subcutaneous tissue of the back. The material to be inoculated was derived from an adenocarcinoma (R. I. 33) which shows numerous spirochetes in sections impregnated by the Levaditi method. In the search for spirochetes the dark field was usually employed, though Giemsa's and Fontana's stains, Loeffler's flagella stain, and India ink preparations were also tried. Peritoneal fluid, blood, smears and fresh teased preparations of normal and carcinomatous tissue, and sections impregnated by the Levaditi method were examined.

The spirochetes prevalent in our mice are most numerous in the peritoneal fluid, where hundreds could sometimes be seen. They were also present in the blood, and occasionally in the pleural cavity. Their average length is 4 to 6 microns, though some measure only 2.7 and others as long as 7.3 microns; both of these extremes however are met but rarely. The number of gyrations is usually 4–5; some have only 3, and the longest may have as many as 9 gyrations. The spirochetes show a very marked motility, which seems greater in the blood than in the peritoneal fluid. They all have a broad flagellum on each end which is plainly visible by dark field illumination and sometimes seems closely connected with a periblast-like structure. The spirochetes can be demonstrated in smears without difficulty with aniline dyes; the flagellae can be stained by Loeffler's flagella method, but do not appear to be demonstrable with the Giemsa stain. India ink preparations show the flagellae plainly, but they are not stained in sections impregnated by the Levaditi method. The spirochetes in these sections resemble closely those described by Gaylord and Calkins and, I have no doubt, are identical with them. These authors were unable to stain the spirochetes by the Giemsa method but their lack of success is easily accounted for by the fact that they tried to stain the organisms in sections. Those now being described cannot be stained in

sections by the Giemsa method either, though they take this stain readily in smears.

The spirochetes can be transmitted to other mice subcutaneously and intraperitoneally without difficulty. This makes it probable that Deetjen saw the same spirochetes; and the probability is supported further by the fact that Deetjen's spirochetes were about the same size, had flagellae, and were prevalent in immune and non-immune laboratory mice. The description given by Wenyon and Borrel does not exclude the possibility that they also were dealing with the same organism. Whether this is closely related to the *Spirocheta morsus muris* (Futaki), which is pathogenic for men and with which it corresponds in several respects (staining properties and flagellae) can not yet be decided. The comparative results of cultural experiments and of agglutination and immunization tests must be awaited.

Numerous spirochetes were found in all mice inoculated with tumor, whether they were immune or not. To discover the source of the spirochetal infection, 46 healthy spirochete-free mice were inoculated with tumor, twenty spirochete-free mice being used as controls. Forty-three of those inoculated (93.5 per cent) showed spirochetes in the peritoneal fluid and in the blood after four to ten days. There was no distinction between successfully and unsuccessfully inoculated mice. All the developing tumors contained spirochetes. Of the control animals, only two showed single spirochetes in the peritoneal fluid after nine days. The result of this experiment points strongly to tumor inoculation as the cause of the infection.

However, another possibility had to be considered; namely, that the spirochetes might live in the superficial skin lesions caused by those parasites of the skin which are so prevalent among mice. With the instrument used for inoculation they might then be deposited in the subcutaneous tissues and so cause a spirochetosis. A sterile trocar was therefore introduced empty into the subcutaneous tissue of 18 spirochete-free mice and immediately withdrawn. But in none of these mice could spirochetes be detected, even after weeks.

As mentioned above two control mice showed spirochetes after nine days' observation, and one of the numerous bugs (*Acanthia lectularia*) infesting the cages therefore came under suspicion as the transmitting agent. A number of these insects were accordingly caught and fed with blood containing spirochetes, and after various intervals (five days to four weeks) allowed to feed on healthy mice. No spirochetes could be found during an observation period of four weeks, but all the mice of this experiment were then unfortunately lost, except one. This remaining one showed spirochetes two months after exposure to the bugs.

These experiments prove that it is inoculation with tumor R. I. 33 which is the almost exclusive cause of the presence of spirochetes in our tumor-bearing mice.

What, then, is the relation between these spirochetes and the tumor? Both Gaylord and Calkins suspected them to be the causative agent,

and point to their regular prevalence in transplanted and sometimes in primary tumors, and to the fact that they are always most numerous at the margin of the tumor, where probably the most intensive cell-proliferation is in progress. On the other hand, it is not without significance that the same spirochete is as common in immune mice, in which transplants do not develop; and it is further to be considered that spirochetes can be transmitted to other mice subcutaneously and peritoneally without difficulty. It was therefore important to investigate the prevalence of spirochetosis among non-inoculated mice. Deetjen, who examined 25 healthy mice, found no spirochetes, and Gaylord and Calkins examined a small number of normal mice, also with negative results. Only Breindl and Kinghorn report that they found two mice infected with spirochetes among six wild mice. I have examined 324 normal mice of our laboratory stock. In six of them (1.85 per cent) spirochetes could be found in the blood, in the peritoneal fluid, and in the subcutaneous tissue. Twenty mice with primary tumors showed no infection, and their tumors also were free from spirochetes. This prevalence among normal mice, together with the fact that the spirochetes can be transmitted experimentally, strengthens the view that they are not an etiological factor in tumor formation, but only an accidental contamination.

Deetjen, who favored this view, attempted to separate the spirochetes from the tumor, but was not successful. I have also tried to do this with intravenous injections of Diarsenol. The idea was to kill all spirochetes at once by a sufficient intravenous dose and so to make the tumor free from spirochetes—that is, to attempt Ehrlich's *therapia sterilisans magna*. The attempt was successful. Twenty-six immune and non-immune inoculated mice were injected in the tail vein with a 1 per cent Diarsenol solution, 0.2 to 0.26 cc. being found sufficient; higher doses usually caused severe collapse. All the mice treated in this way remained permanently free from spirochetes. No effect upon the existing tumors could be observed, for they continued to grow as before; but they were all free from spirochetes. These tumors, made sterile by Diarseonol, were now inoculated into 43 spirochete-free mice. Twenty-five (58 per cent) of these developed tumors, but none of the mice and none of the tumors contained spirochetes. Tumors growing in this batch of mice were again transplanted into 44 spirochete-free mice, of which 29 (66 per cent) proved susceptible. All mice in this second generation after the Diarsenol injection remained free from spirochetes, as did the tumors. These experiments prove definitely that there is no etiological relation between spirochetes and tumor.

We have apparently to deal with a widespread spirochetal infection among mice. When a primary or transplanted tumor develops in an infected mouse, the spirochetes living in all the body fluids are transported in larger or smaller numbers, according to the blood supply, into the tumor. This explains their great prevalence at the margin, where there are most blood-vessels. They are then inoculated together with the tumor into other mice, and as all mice are susceptible they give rise to a general spirochetosis.

That this actually does happen is proved by the following experiment. Fifteen mice were infected by the intraperitoneal injection of blood and peritoneal fluid containing spirochetes, and four days later inoculated with one of those tumors which had remained free of spirochetes through two generations by reason of the Diarsenol treatment previously referred to. Four mice were lost before a secondary tumor had time to appear, but six (53 per cent) of the remaining mice developed growths. As expected, all these mice showed spirochetes, and the organisms could be found also in all the tumors. These tumors, now containing spirochetes, were inoculated into 13 spirochete-free mice, with the result that 9 became infected and 6 (46 per cent) developed tumors; of these 6 growths, the 4 that could be examined showed spirochetes.

It is interesting to note the change in the virulence of the tumor during these experiments. The untreated tumor gives about 80 per cent takes in our routine transplantations; the spirochete-free tumor taken from the Diarsenol mice produced, in the first generation, only 58 per cent, and in the second 66 per cent successful inoculations. After the tumor had been reinfected with spirochetes, its virulence decreased to 54 per cent in the first, and 46 per cent in the second generation. The tumor, therefore, had lost almost half its virulence, probably because the vitality of its cells had been impaired by the Diarsenol treatment of their host and the reinfection with spirochetes. But experiments on a much larger basis are of course necessary, to throw more light on this question.

SUMMARY

1. All our mice inoculated with tumor R. I. 33 had spirochetes in the blood, peritoneal and pleural fluids, in the subcutaneous tissue, and in the tumors. The spirochetes can be transmitted to other mice intraperitoneally or subcutaneously without difficulty.

2. A similar spirochete can also be found in almost 2 per cent of normal mice.

3. The spirochetes are not an etiological agent, but merely a contamination of this tumor.

4. The spirochetes can be separated from the tumor and re-introduced into it.

5. The virulence of the tumor has decreased from 80 per cent to 46 per cent "takes" during these experiments.

Dr. Clowes: It is interesting to note that the Diarsenol used in the experiments reported by Dr. Mueller was employed in a dosage considerably lower than that required to kill the mice. It appears to possess a remarkable penetrating power. The fall in the virulence of the tumor corresponds with our observations, made some years ago, regarding the influence of potassium cyanide in destroying bacteria in tumors. It was found possible to destroy bacteria and yet leave the tumor sterile, so that it could be transplanted; but its virulence was greatly impaired.

THE EFFECTS OF X-RAY IRRADIATION ON LIVING CARCINOMA AND SARCOMA CELLS IN TISSUE CULTURES IN VITRO[1]

NORIYOSHI KIMURA

From the Pathological Laboratory of the University of Chicago and the Otho S. A. Sprague Memorial Institute

Received for publication, January 28, 1918

During the years which have passed since the method of tissue culture in vitro was commenced by Harrison, and modified by Carrel and Burrows (4), very little attention has been paid the investigation of the biological effects of *x*-rays upon cells growing in culture in vitro.

Contamin (7) exposed mouse carcinoma to *x*-rays under various experimental conditions. From the result of a number of his observations, he came to the conclusion that the younger the tumors, the more sensitive they were to *x*-rays, and that the disappearance of a large tumor under *x*-rays caused the death of the animal, probably by intoxication. In other experiments made by Contamin (18) with Nogier and Jaubert de Beaujeu, extirpated mouse carcinoma was exposed to *x*-rays and then inoculated into normal mice. They concluded that the action of *x*-rays on extirpated tumor cells hinders their subsequent growth in the animal body.

Clunet (5) and Raulot-Lapointe treated malignant tumors in situ with *x*-rays and studied them histologically at various stages of the treatment. They found that the squamous carcinoma cells in the human subject finally disappeared, passing through five successive stages from the latent phase to the formation of the connective tissue scar. With sarcomatous growths, Clunet and Raulot-Lapointe found that the latent phase was much shorter than in the other types of malignant growths.

[1] The author has not read the proof of this article.

Wedd and Russ (31) reported a series of experiments in which a transplantable mouse carcinoma was removed from the animal in which it had grown, kept between mica sheets during the exposure to radium rays, and then inoculated into normal mice. It was found that no growth resulted from grafts which were exposed a sufficient length of time.

Russ and H. Chambers (25) reported an observation made with Jensen's sarcoma. The authors concluded that the tumor cells irradiated by β-rays of radium (1.63 mgm. per square centimeter for ninety-six minutes) or radium emanation of 0.53 millicuries per cubic centimeter for forty-five minutes, did not produce tumors after inoculation into normal mice, though they showed no histological changes in the cells.

Wassermann (30) exposed extirpated cancerous tissue to radium rays from mesothorium, and then inoculated it into animals, no tumor resulting. He supposed that the multiplication and cell division were affected by the rays while the nutrition of the cells remained uninfluenced, though he did not try cultures in vitro.

C. Price Jones (12) exposed mouse carcinoma and mouse sarcoma to radium rays, made tissue cultures of them, and found that the mitotic division of cells was inhibited, though their spreading growth was not retarded.

Prime (23) has recently worked in the same direction, reporting that a carefully measured amount of radium rays injured the power of mitotic cell division in the tissue, and that the inoculation of a sufficiently irradiated tumor produced no growths in mice.

In all these articles, however, no observations seem to have been made on the effects of x-rays upon living cells growing in plasma outside of the body. The present experiments were undertaken, therefore, to clear up the question of the biological effect of x-rays on tumor cells, and to determine what dose of x-rays would render tumor cells incapable of producing tumors when subsequently inoculated into animals.

PREPARATION OF THE CULTURE MEDIA

A small glass tube (*A* in text-fig. 1) was coated thickly with paraffin and inserted into an aluminum centrifuge tube (*B* in

Text fig. 1. Tube in which plasma is obtained.

text-fig. 1) stopped by means of a cork. The space between tubes *A* and *B* was filled with ice and salt mixture, and the whole apparatus kept in a glass with ice mixture until ready for use.

Under ether anesthesia the carotid artery of a guinea-pig was exposed about 2 to 3 cm. The distal part was ligatured by a thread while the proximal part was fixed by a small artery clamp to stop the blood stream. Proximal to the ligature the artery was pinched by a small clamp without narrowing the lumen.

Between this and the ligature the artery was cut off sharply. After the proximal clamp had been released, the blood was allowed to flow directly into the tube A, which was kept cool in ice mixture. When the blood was filled up to one-third the height of the tube A, the artery was clamped again. The tube with blood was promptly and powerfully centrifugalized for three minutes. The supernatant plasma (text-fig. 1) was separated by a small pipette into four or five small tubes, which were coated thickly with paraffin and prepared in ice mixture. It was necessary neither to keep the pipette cool nor to coat it with paraffin. These tubes which held the plasma were plugged with sterilized cotton and covered with tinfoil in order to protect them from contamination or drying, and preserved in a frozen condition in a Universal jar filled with ice mixture. The greatest difficulties encountered were obtaining a sufficient amount of mouse blood and keeping the plasma from coagulating. The mouse was prepared by the removal of the hair from the throat region, and the skin rendered sterile by iodin. The skin over the thyroid region was picked up with a forceps and cut off with scissors. The thyroid gland was picked up and bluntly loosened from the under layer without injuring the tissues. The carotid artery appeared on both sides in the bottom of the space made by picking up the gland. After the artery had been cut, the escaping blood was collected in the paraffin coated tube (text fig. 1) in ice mixture. In the same manner as described already, the tube was immediately centrifugalized for three minutes. The supernatant plasma was drawn up and put into cold tubes in ice mixture.

It was found very easy to obtain chicken plasma. A heavy syringe needle, sterilized by boiling, was inserted directly into the wing vein and the blood was collected drop by drop in an ice cold

paraffin coated tube, and centrifugalized in the same way as above. The supernatant plasma was drawn off into tubes.

The plasma thus obtained from guinea-pigs, mice, and chickens, did not lose its coagulability for about ten to fourteen days as it was kept in ice mixture, and remained fluid for hours during each series of experiments, when it was kept cool in ice, and at no time did coagulation take place before the completion of the experiment. One tube containing 0.2 to 0.3 cc. of plasma was enough for each series of experiments.

MATERIAL AND TECHNIQUE OF CULTURE

The tumors used were mouse carcinoma R. T. 33, which had been propagated for three years at the State Institute for the Study of Malignant Disease, Buffalo, New York. These tumors, for which I am indebted to Dr. Gaylord, showed a type of adenocarcinoma (fig. 2) and with the mice which I had inoculated gave "takes" in about 90 per cent of implants. Another tumor, for which I am indebted to Dr. F. C. Wood of the Crocker Fund, was the Ehrlich mouse sarcoma (fig. 3), which had been under observation for some years in the Crocker Laboratory in New York, and gave approximately 100 per cent of successful inoculations in Chicago mice.

The tumors, after reaching a full growth, were cut out strictly aseptically in Ringer's solution and cut into many pieces of equal size. They were then put into three or four small sterilized glass tubes with a diameter of 1 cc. and 1.5 cc. in depth. Each of these glass tubes was stopped with a cork pushed in over a sheet of sterile Japanese paper in order to prevent any contamination that might occur during the exposure of the x-rays. After the removal of the cork stopper, the pieces of tumor, which were kept in these tubes with Ringer's solution, were exposed to x-rays merely through the layer of Japanese paper at a distance of 2 cm. from the x-ray bulb. In this manner each piece of tumor was exposed for a certain required length of time without other obstacles between the bulb and the tumor pieces. Another factor in favor of this method was the fact that the tissues did

not begin to grow while exposed to the ray as was found to be the case when they were planted first into the plasma and then exposed.

The ray used for this series of experiments was of a moderately soft type, the spark gaps ranging from 4 to 8 cm.; the length of time for exposure varied from five to thirty-six minutes. The amount of the effective ray was measured by Hampson's radiometer. The initial tint is the color of the unexposed pastille and the sixteenth change represents the browner shade of color, equivalent to the maximum or B tint of the Sabouraud's pastille. The terms E.1, E.4, E.8, or E.12 in the following experiments indicate that the Hampson's pastille used showed no. 1, no. 4, no. 8 or no. 12 tint, that is, equivalent to a dose $\frac{1}{16}$, $\frac{1}{4}$, $\frac{1}{2}$, or $\frac{3}{4}$ of Sabouraud's B tint.

The irradiated tissue was removed to a watch glass with Ringer's solution and was cut into fine fragments. Each fragment was transferred to a cover glass by a small pipette provided with suction bulb. The excess Ringer's solution was sucked up with the same pipette. Some small drops of plasma were added immeiately to the tissue fragment, then some drops of mouse serum with Ringer's solution were added. The plasma and serum were mixed up and spread around the fragment with a pointed cataract knife. The cover slip, which previously was ringed with vaseline on all its edges, was then inverted over a fairly deep hollow ground slide. The cover slip was then sealed with molten paraffin around its edges. An equal number of control cultures from an irradiated tissue was made in each series. The slide preparations were incubated at 37°C. and microscopic observation was made every twelve to twenty-four hours.

To obtain the stained specimen the cover slips, mounted with growing culture, were put into 10 per cent formalin for twenty-four hours or more, then they were washed in water for one hour and stained with diluted Delafield's haematoxylin for one to two hours, and decolorized for about thirty minutes in water, to which a few drops of diluted hydrochloric acid was added. The specimens were washed again in water until a violet blue color developed. It is important to decolorize the over-stained plasma and

tissue as much as possible, otherwise no good specimen was obtained.

The tissue piece irradiated with certain required doses of x-ray in Ringer's solution was divided into many fragments, each of about 20 mgm., and these were inoculated into the right axilla of a number of normal mice. An equal number of tissue fragments from the control tissue were inoculated into the other axilla of the same mice. The observations respecting the rate and course of the grafts planted into the animals follows.

The culture media used for the experiments were: Mouse plasma, guinea-pig plasma, chicken plasma, guinea-pig plasma plus mouse serum diluted with Ringer's fluid, chicken plasma plus mouse serum diluted with Ringer's fluid.

In comparing all these media used for the culture of the tumor, it was found that the mouse plasma (homogenous and autogenous) or a mixture of guinea-pig plasma and mouse serum diluted with Ringer's solution were most satisfactory as media for the culture of the tumors. There was no noticeable difference in either case, whether the mouse plasma or the guinea-pig plasma with diluted mouse serum was used. Because the guinea-pig plasma can be obtained much more easily and in greater quantity than mouse plasma, most of the cultures, except a few specimens, were made with guinea-pig plasma, to which was added the mouse serum and Ringer's solution.

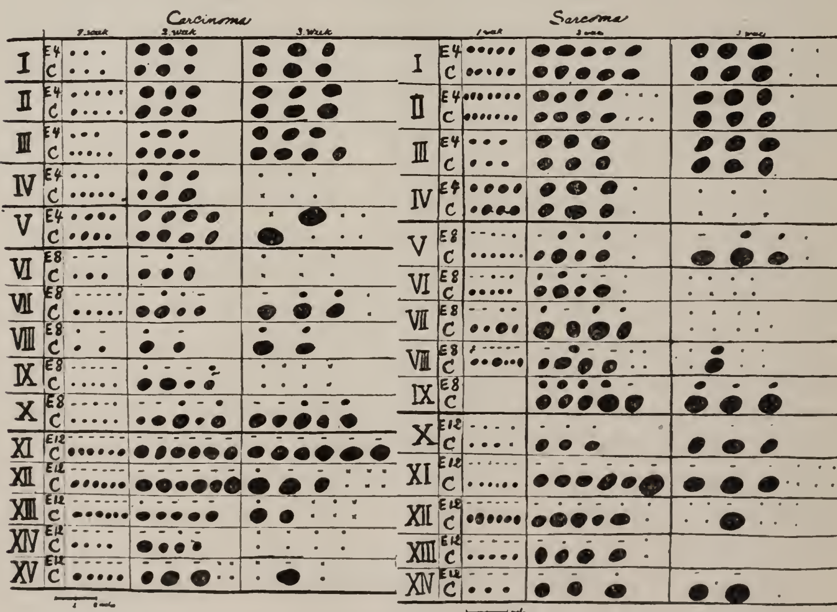
RESULTS OF EXPERIMENTS

Mouse carcinoma

Mouse carcinoma, cultivated under the conditions described above in respect to technique, showed but very little growth the first twenty-four hours in most cases of all series, both in the control and in the exposed tissue. After twenty-four hours of incubation, the original fragment of tissue became thinner and somewhat translucent, especially on the edge of the fragments, from which the cells spread out into the plasma media. The cells did not migrate separately into the media, but merely formed cell groups composed of a number of cells. The boundaries of each cell were indistinct.

Many of the cells on the advancing edge formed pseudopodia. There were many cells of different morphological type which supposedly originated from the stroma. Cells of this type were present in some specimens, while others showed no such type of cells.

Most cultures reached the maximum growth in forty-eight to ninety-six hours after incubation. Karyokinetic figures were observed in the cells in the growing zone in stained specimens



Text fig. 6. No difference between irradiated (E.4 dose) and control tumor, after inoculation.

from the control tissue (fig. 4). The specimen from the tumor piece exposed to E.1 of rays, showed no difference from the control in growth in the plasma media during the whole observation, and mitotic figures of growing cells were seen frequently in the stained preparations in all stages. Both the tissues, control and irradiated, produced good sized tumors after inoculation into normal mice.

In cultures from the tissue exposed to E.4 of the ray, the proliferation of cells was just as extensive as in the specimen made from control tissue (figs. 9, 10), while the spreading-out of the thread-like cells, which supposedly originated from stroma, showed itself a little more active than in the control. The mitotic figures were found as abundant as in control cultures (fig. 5).

No difference was seen in speed and rate of growing tumors, produced by inoculation of either the treated or the control piece, as is shown by the preceding chart (text-fig. 6) and by table 1.

TABLE 1

Carcinoma

	NUMBER OF "TAKES"		PER CENT	NUMBER OF "TAKES"		PER CENT	NUMBER OF "TAKES"		PER CENT
	E.4	Control		E.8	Control		E.12	Control	
First week	19	19	100	3	19	16.0	0	26	0
Second week	16	16	100	8	18	44.5	0	24	0
Third week	10	10	100	6	10	60.0	0	12	0

In the unstained culture, when E.8 doses of x-ray were given, the proliferation of cells growing in groups showed almost the same extension as did the specimen of control tissue (fig. 11). In some specimens, however, the cells of the stroma type, which spread out like fibrillae into the plasma media, seemed more vigorous than those in the control specimens. But in the stained specimen a few mitotic figures were found in most of the specimens cultivated from the irradiated tissue (fig. 7), while many more mitotic figures appeared in the control specimen (fig. 4). After the inoculation into mice, it was found that the treated tissue gave "takes" of 16 per cent in the first, 44.5 per cent in the second, and 60 per cent in the third week, according to table I, while the control tissue produced tumors in 100 per cent of the grafts.

All the tumors grown in mice from the treated cells, however, reached only one-third of the size of those from the control inoculations.

In the cultures of the tissue which was exposed to E.12 of the active ray, it was found that after forty-eight hours there was still a marked outgrowth of cells (fig. 12). The same results were found in the control specimen. None of the stained specimens of the exposed tissues showed mitotic figures in growing cells (fig. 8).

The inoculation of the treated tissue into mice produced in the first week no tumor in 27 cases in 6 series. In the second and third weeks two nodules were developed to the size of a rice grain; they, however, did not grow further, but disappeared.

Mouse sarcoma

In the cultures made in guinea-pig plasma diluted with mouse serum, the margin of the fragment became gradually sharp and opaque. In five to twelve hours a few round cells had emigrated into the plasma media and a few irregular cells began to grow out radially from the edges of the fragment, which began to have a serrated appearance. The individual cells formed many pseudopodia, which were seen especially in the advancing side of the cell body. The number of cells emigrating into the media increased more rapidly than those in the culture of carcinoma tissue. After forty-eight hours the original fragment became more translucent than it was before, and it was surrounded with thick layers of growing cells, which were rich in protoplasm. When the stained specimens were fixed, at the end of twenty-four to seventy-two hours, they exhibited the presence of numerous mitotic figures in the outgrowing cells (fig. 13).

The specimen from the tissue exposed to E.1 of x-ray showed no difference in respect to the rate of growth from that of the control tissue (fig. 17). The inoculation of the treated piece into mice produced tumors as large as those of the control tissue.

In the culture of the tissue exposed to E.4, the proliferation of cells was found to be more vigorous than that in the control cultures (fig. 18). In the stained preparations, however, the number of mitotic figures of cells was about equal to that in the control specimen (fig. 14). After inoculation into mice, the

tumors produced from irradiated cells in the first week seemed to grow a little faster than those in controls; but in the second and the third week there was no difference either in speed or size, and the inoculation gave "takes" of 100 per cent.

In the unstained cultures from the tissue which was exposed to E.8, the proliferation of cells and the area of growth were similar to the proliferation in the control specimen (fig. 19). But the mitotic figures in the stained specimen diminished greatly in number compared with those in the control specimen (fig. 15). In the first week after inoculation, the grafts gave "takes" of 35 per cent, 62 per cent in the second, and 85.7 per cent in the third week. These tumors, however, developed only to one-third the size of those in the controls (text-fig. 6).

TABLE 2

Sarcoma

	NUMBER OF "TAKES"		PER CENT	NUMBER OF "TAKES"		PER CENT	NUMBER OF "TAKES"		PER CENT
	E.4	Control		E.8	Control		E.12	Control	
First week	19	19	100	7	20	35.0	0	24	0
Second week	15	15	100	10	16	62.5	0	21	0
Third week	9	9	100	6	7	85.7	0	9	0

In the cultures made from the tissue which had been irradiated to E.12, it was found that there was still a marked outgrowth of cells as well as in the control specimen (fig. 20). No mitotic figures, however, were found in the stained specimen (fig. 16). None of the inoculated grafts into 23 mice in 5 series produced any tumor the first week. In the second week there were found 4 hardly perceptible nodules in 21 mice. These, however, did not grow further and disappeared entirely in the third week, as is shown in text-fig. 6 and table 2.

DISCUSSION

In accordance with the results of the experiments described above, the rate of the cell growth in cultures of mouse carcinoma and mouse sarcoma was, after forty-eight to ninety-six hours'

incubation, nearly equal in both the control and the exposed tissues which were x-rayed to E.1–E.12 (figs. 9, 10, 11, 12, 17, 18, 19, 20). The sarcoma, as compared with the carcinoma, however, was always superior in its growth in culture media. After an exposure of E.8, the number of the mitotic figures in the culture of growing tumor cells was diminished to a minimum of 2 to 4 in carcinoma and 2 to 13 in sarcoma cultures. An exposure, however, of E.12, entirely inhibited the mitotic division of cells, and they were never found in the stained specimen, either in carcinoma or in sarcoma (figs. 8, 16). See table 3.

By an exposure of E.4, the sarcoma not only remained without injury to the power of proliferation by mitotic cell division, but the exposed tissue produced tumors in the first week after inoculation —i.e., somewhat earlier than the control tissue did (text-fig. 6). This phenomenon was due to the action of x-rays, to which the tissue was exposed. It seems that the x-ray in this dose acted upon the tissue as a stimulation and temporarily raised the metabolism of the tumor cells. Consequently the cells in the exposed tissue were stimulated to grow more quickly the first week after inoculation than those in the control.

In connection with the process of oxidation in the tumor tissue, I tried some experiments hoping that the effects of x-ray on the living cells might be explained to some extent. The tumor pieces were exposed to x-ray of various doses varying from E.4 to E.12. One piece of control was put in the chamber of one side of Dr. Tashiro's biometer and an irradiated piece in the other chamber of the other side.

Observation was made as to the quantitative difference of CO_2 production in both chambers. The results of these experiments are shown in the record of tables 4 and 5.

In the experiments with the tumor pieces, both in carcinoma and sarcoma, which were exposed to E.4 of x-ray, carbon dioxide production began to appear in ten minutes after the arrangement. The quantity of precipitation by barium hydroxide in the chamber which contained the exposed tissue was greater than that in the chamber which had the control tissue.

TABLE 3

SPECIMEN	NUMBER OF FIGURES	AMOUNT OF X-RAY	LENGTH OF TIME	STAINED SPECIMEN	
				Growth in culture	Number of mitotic figures
Carcinoma					
			minutes		
111	4, 9	Control		Fair	7
4				Fair	15
31				Slight	8
102				Slight	16
110	10, 5	E.4	12	Fair	6
104				Fair	8
101				Slight	4
103				Slight	7
18	11	E.8	24	Fair	2
100				Slight	3
35				Fair	4
28				Slight	4
5	8, 12	E.12	36	Fair	0
33				Slight	0
34				Slight	0
107				Fair	0
Sarcoma					
1	13	Control		Fair	21
95				Fair	40
105				Fair	Numerous
106				Fair	Numerous
43	14, 18	E.4	12	Fair	40
17				Fair	25
52				Fair	31
15				Fair	27
11	15, 19	E.8	24	Fair	10
98				Fair	6
94				Fair	13
50				Fair	2
96	16, 20	E.12	36	Fair	0
41				Fair	0
46				Fair	0
53				Fair	0

On the contrary, in the experiments made with the tissue exposed to E.12 of x-ray, the production of carbon dioxide was less than that of the control.

In the unstained specimen cultured from tissue which was exposed to E.4, the outgrowth of cells seemed somewhat more

TABLE 4

Carcinoma

CO_2 COMPARED				CO_2 COMPARED			
Time	E.12		Control	Time	E.4		Control
	*29 mgm		*27 mgm.		*75 mgm.		*75 mgm.
4.42	– – – – –		– – – – –	2.19	– – – – –		– – – – –
4.50	– – – – –	=	– – – – –	2.29	– – – – –	>	– – – – –
7.00	– – – – –	<	– – – – –	2.39	– – – – –	>	– – – – –
	++		+++	2.49	– – – – –	>	– – – – –
				2.59	+++– –	>	– – – – +
	52 mgm.		50 mgm.		75 mgm.		75 mgm.
7.50	– – – – –	<	– – – – –	2.35	– – – – –		– – – – –
7.55	– – – – –	<	– – – – –	2.45	– – – – –	>	– – – – –
8.00	– – – – –	<	– – – – –	2.55	– – – – –	>	– – – – –
8.10	– – – – –	<	– – – – –	3.05	– – – – –	>	– – – – –
				3.15	– – – – –	>	– – – – –
	++		+++	3.35	– – – – –	>	– – – – –
					++		+
	57 mgm.		55 mgm.		63 mgm.		64 mgm.
7.22	– – – – –		– – – – –	2.13	– – – – –		– – – – –
7.32	– – – – –		– – – – –	2.23	– – – – –	>	– – – – –
8.12	– – – – –	<	– – – – –	2.33	– – – – –	>	– – – – –
8.20	– – – – –	<	– – – – –	2.43	– – – – –	>	– – – – –
8.40	– – – – –	<	– – – – –	3.13	– – – – –	>	– – – – –
	++		+++		+++		++

* The figures are the weights of the pieces of tumor tissue used in each experiment. No quantitative determination of the amount of CO_2 produced was made, but the relative amounts produced by irradiated tumor and control are indicated by the > sign.

extensive than the outgrowth of the control culture, both in sarcoma and carcinoma. But in the stained specimens no noteworthy difference in number of the mitotic figures was seen. This stimulating effect on the sarcoma tissue was more prominent than the effect shown in the culture of the carcinoma. A some-

what similar difference of the stimulating effect of x-ray E.4 on the tissues appeared prominently in the inoculation experiments of sarcoma, but the carcinoma was not much stimulated by the rays and consequently the power of proliferation was diminished

TABLE 5

Sarcoma

CO_2 COMPARED				CO_2 COMPARED			
Time	E.12		Control	Time	E.4		Control
	*74 mgm.		*70 mgm.		*132 mgm.		*132 mgm.
2.25	------		------	3.43	------		------
2.40	------	=	------	3.53	------	>	------
2.45	------	<	------	4.03	------	>	------
3.00	------	<	------	4.43	------	>	------
3.10	------	<	------				
3.25	------	<	------		++++		+++
	++		+++				
	22 mgm.		16 mgm.		132 mgm.		132 mgm.
2.36	------		------	4.13	------		------
2.46	------	<	------	4.23	------	>	------
2.56	------	<	------	4.33	------	>	------
	+		++	5.13	------	>	------
					++++		+++
	48 mgm.		47 mgm.		85 mgm.		85 mgm.
3.38	------		------	6.33	------		------
3.48	------	<	------	6.43	------	>	------
3.58	------	<	------	6.53	------	>	------
4.08	------	<	------	7.03	------	>	------
4.18	------	<	------				
4.28	------	<	------		++		+
	+		+++				

* The figures are the weights of the pieces of tumor tissue used in each experiment. No quantitative determination of the amount of CO_2 produced was made, but the relative amounts produced by irradiated tumor and control are indicated by the > sign.

gradually without any preliminary stimulation (text-fig. 6 and table 4).

The fact that the number of mitotic figures in culture and the quickness of growth of the grafts inoculated into mice increased to some extent when the tissues previously were exposed to E.4

of rays, and the fact that both decreased gradually when the tissues were irradiated to E.8–E.12, coincide with the results of the experiments on the oxidation of the tumor tissues.

For the explanation of the presence of only a few mitotic figures in the culture and retardation of the growth of the grafts when the tissue was exposed to E.8, the reports made by Bordier and others are to be taken into consideration. We know that the younger the cell generation the greater the radio-sensibility of the living protoplasm; and consequently the younger neoplastic cells are most sensitive, while the cells in the latent stage are less sensitive to the *x*-ray action. Hence the mitotic figures seen in the tissues that had been exposed to E.8 supposedly originated from some resting cells in the fragments, in which they remained without an intensive effect of *x*-ray and produced the further division in culture in vitro or developed to a tumor in mice, though their mitotic proliferation was greatly retarded.

On the contrary, all the cells in the specimen exposed to E.12 were sufficiently damaged by the ray action, and consequently the dividing process of the cell chromosomes had ceased, and the fragments did not grow any more to a tumor in mice.

CONCLUSIONS

1. The mouse carcinoma and sarcoma grow as well in guinea-pig plasma to which has been added mouse serum diluted with Ringer's solution, as in mouse plasma itself.

2. The culture growths of carcinoma and sarcoma from mice showed each the characteristics of the original tissues. Sarcoma produced a radial outgrowth spreading widely into the plasma media, while the carcinoma cells grew continuously into the media, as cell groups, from the edges of the fragments.

3. The outspreading growth of cells in culture, both sarcoma and carcinoma, was not stopped by *x*-ray action varying from E.4 to E.12. The mitotic figures of cells were limited to a minimum after an exposure of E.8 (one-half dose of Sabouraud's B tint). After exposure to E.12 (three-fourth dose of Sabouraud's B tint), however, they disappeared entirely, and the treated tissue produced no tumor when inoculated into mice.

4. The growing power of sarcoma after E.4 exposure was stimulated to some extent, while carcinoma was not appreciably influenced. An exposure of tissues to E.12, both sarcoma and carcinoma, stopped the growing power of these tissues when inoculated into mice, and eliminated the process of mitotic division of cells.

5. The process of oxidation of tissues, both sarcoma and carcinoma, was stimulated by the x-ray action of E.4 and retarded by exposure to E.12 of the ray.

REFERENCES

(1) Apolant: Deutsch. med. Wchnschr., 1904, xxx, 454.
(2) Bordier: Fortschr. a. d. Geb. d. Röntgenstrahlen, 1913, xx, 202.
(3) Beckton: Arch. Middlesex Hosp., 1914, xxxiii, 123.
(4) Carrel and Burrows: Jour. Exper. Med., 1911, xiii, 387 and 571.
(5) Clunet and Raulot-Lapointe: Recherches Expérimentales sur les Tumeurs Malignes, Paris, 1910.
(6) Colwell and Russ: Radium, X-rays and the Living Cell, London, 1915.
(7) Contamin: Compt. rend. Acad. d. sc., 1910, cl, 1537.
(8) Ebeling: Jour. Exper. Med., 1914, xx, 130.
(9) Heimann: Berl. klin. Wchnschr., 1914, li, 1516.
(10) Heineke: München. med. Wchnschr., 1913, lx, 2657.
(11) Ingebrigsten: Jour. Exper. Med., 1912, xvi, 421.
(12) Jones and Mottram: Arch. Middlesex Hosp., 1914, xxxiii, 21.
(13) Lambert and Hanes: Jour. Exper. Med., 1911, xiii, 495; 1911, xiv, 129; 1913, xviii, 406; 1914, xix, 398.
(14) Lambert: Anat. Rec., 1912, vi, 91.
(15) Lambert and Hanes: Virchows Arch., 1913, ccxi, 89.
(16) Lambert: Jour. Cancer Research, 1916, i, 169.
(17) Loeb: Anat. Rec., 1912, vi, 109.
(18) Contamin, Nogier, and Jaubert de Beaujeu: Compt. rend. Acad. d. sc., 1909, cxlix, 1398.
(19) Oberndorfer: Zentralbl. f. allg. Path. u. path. Anat., 1914, xxv, 411.
(20) Packard: Jour. Exper. Zool., 1915, xix, 323; 1915, xxi, 199.
(21) Petry: Biochem. Ztschr., 1913, lvi, 341.
(22) Pentimalli: Ziegler's Beiträge, 1914, lix, 674.
(23) Prime: Jour. Cancer Research, 1917, ii, 107.
(24) Richards: Am. Jour .Roentgenol., 1915, ii, 908.
(25) Russ and Chambers: Proc. Roy. Soc. Med., London, 1913, vi, 62–68.
(26) Schwarz: München. med. Wchnschr., 1913, lx, 2165.
(27) Tashiro: Chemical Signs of Life, University of Chicago Press, 1917.
(28) Walton: Jour. Exper. Med., 1914, xix, 121; 1914, xx, 554.
(29) Walton: Proc. Roy. Soc., London, 1914, lxxxvii, Series B, 452.
(30) Wassermann: Deutsch. med. Wchnschr., 1914, xl, 524.
(31) Wedd and Russ: Jour. Path. and Bacteriol., 1912, xvii, 1.
(32) Wermel: München. med. Wchnschr., 1914, lxi, 299.

PLATE 1

Fig. 2. Stained section of original carcinoma, many mitotic figures. × 275.

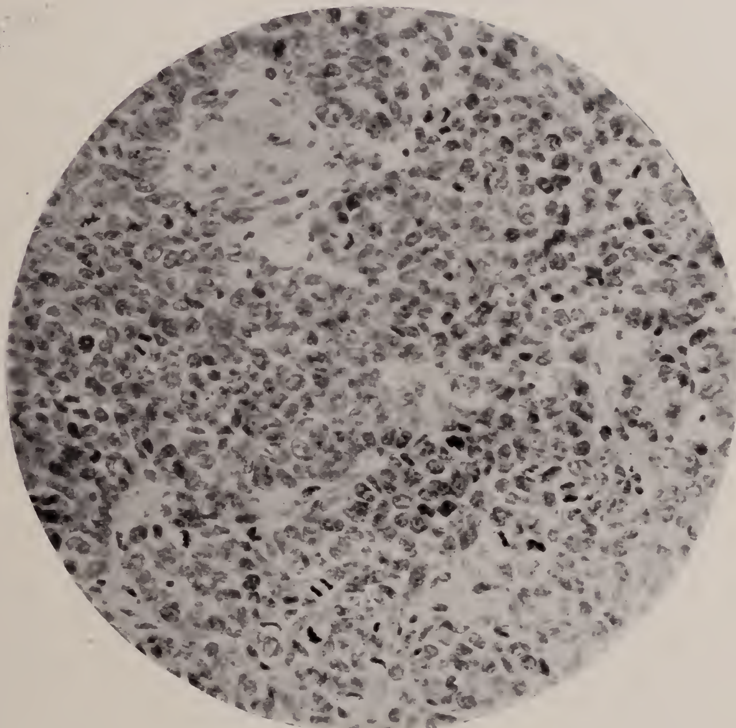


PLATE 2

Fig. 3. Stained section of original sarcoma, many mitotic figures. $\times$ 275.

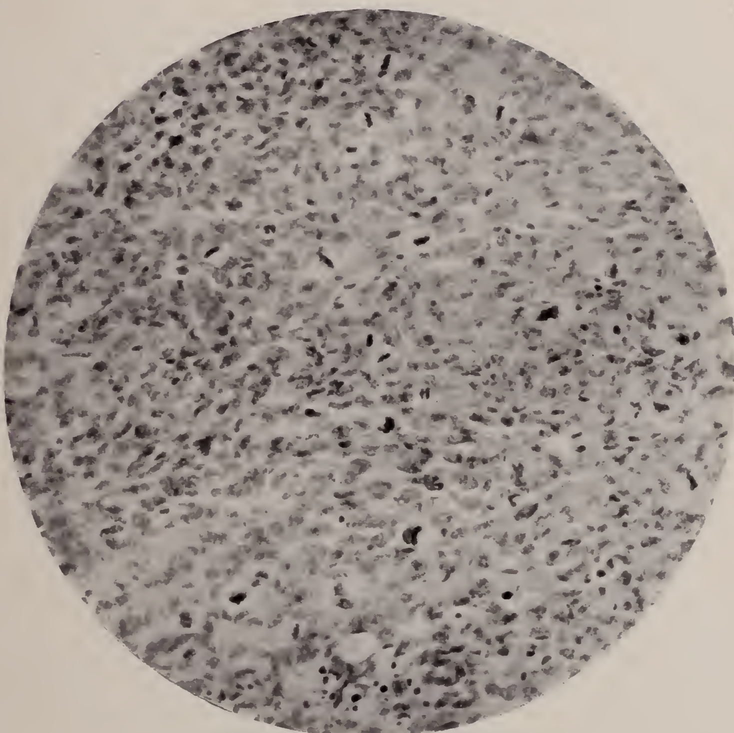


PLATE 3

Fig. 4. Tissue culture, control, carcinoma, 2 mitotic figures. $\times$ 275.

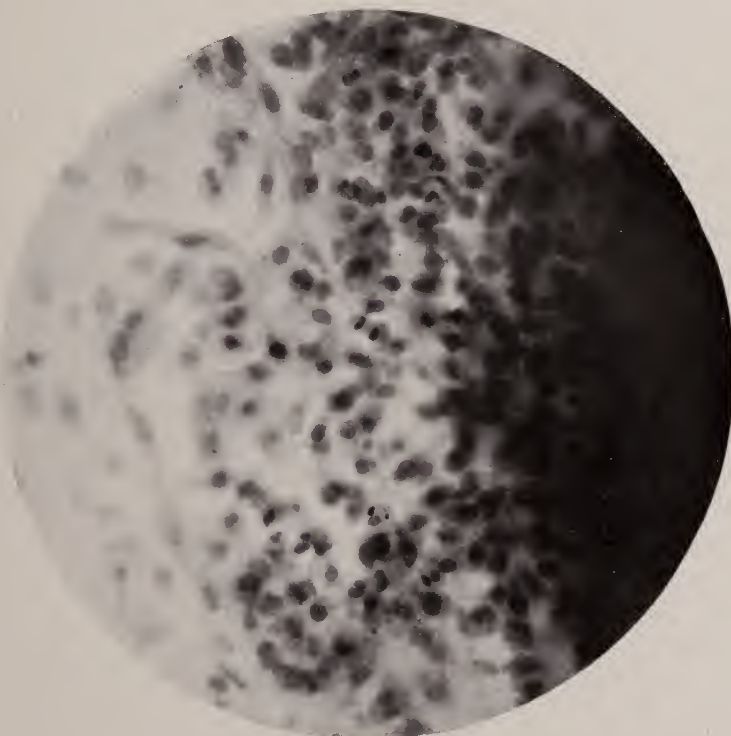


PLATE 4

Fig. 5. Tissue culture, experiment 4, carcinoma, 1 mitotic figure. $\times$ 275.

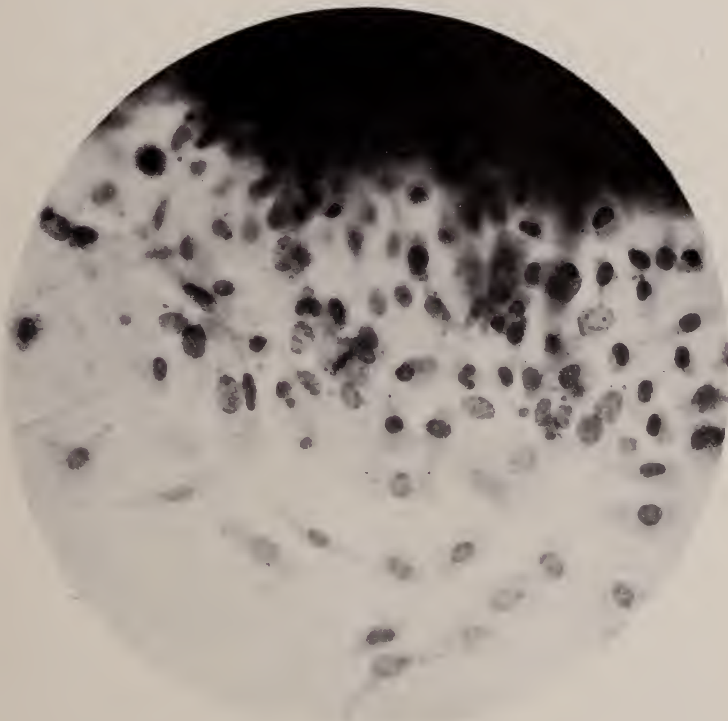


PLATE 5

Fig. 7. Tissue culture, experiment 8, carcinoma, 1 mitotic figure. $\times$ 275.

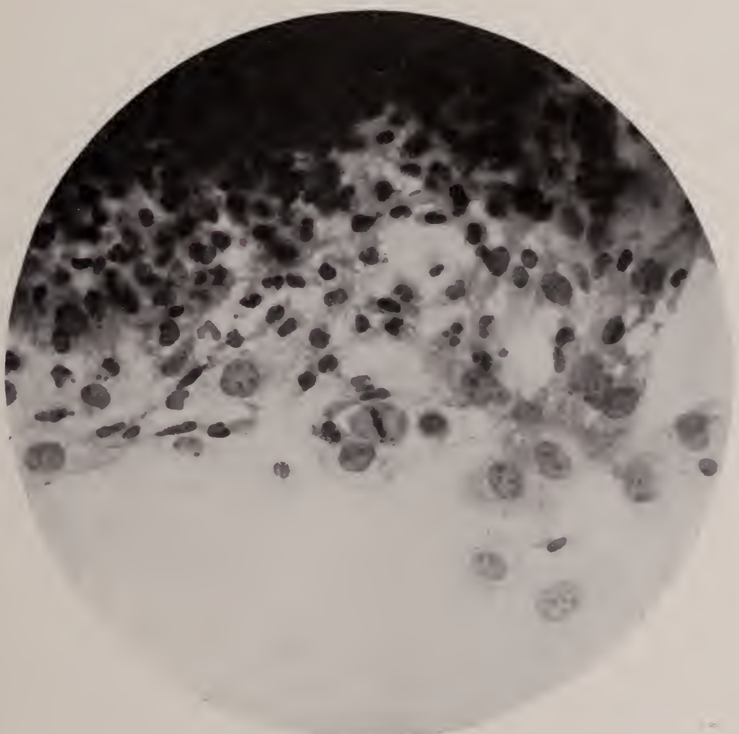


PLATE 6

Fig. 8. Tissue culture, experiment 12, carcinoma, no mitotic figure. $\times$ 275.

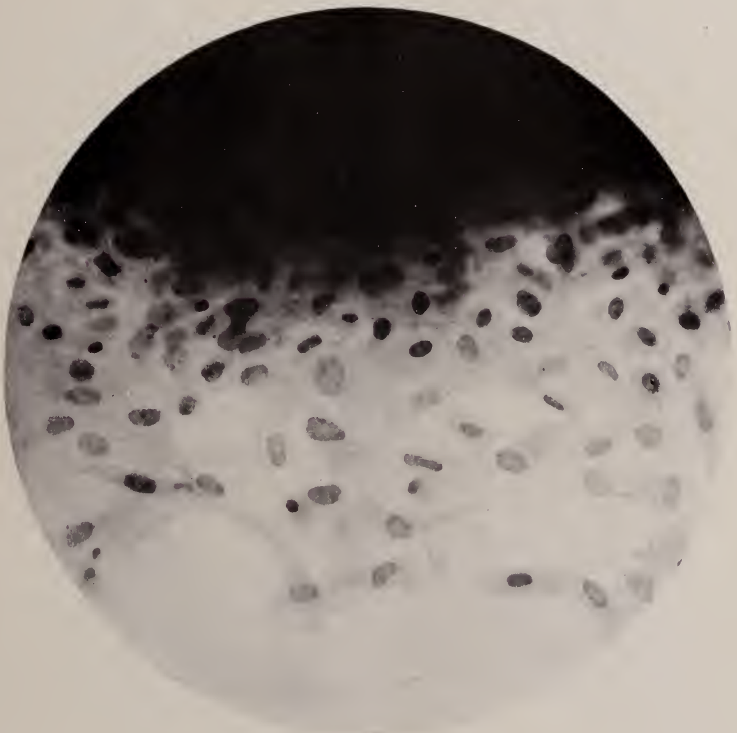


PLATE 7

Fig. 9. Tissue culture, control, carcinoma. $\times$ 37.
Fig. 10. Tissue culture, experiment 4, carcinoma. $\times$ 37.
Fig. 11. Tissue culture, experiment 8, carcinoma. $\times$ 37.
Fig. 12. Tissue culture, experiment 12, carcinoma. $\times$ 37.

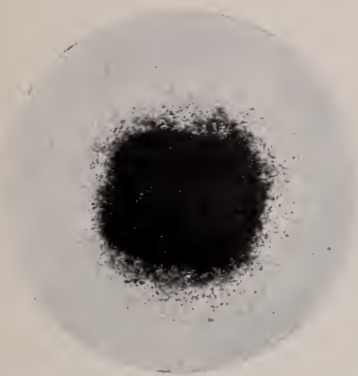


FIG. 9

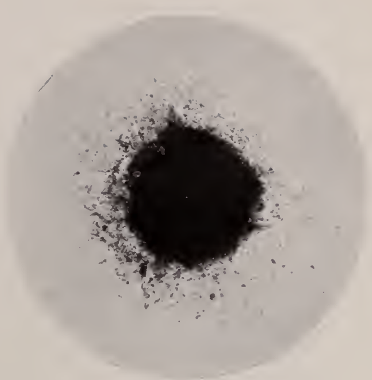


FIG. 10

FIG. 11

FIG. 12

PLATE 8

Fig. 13. Tissue culture, control, sarcoma, 2 mitotic figures. $\times$ 275.

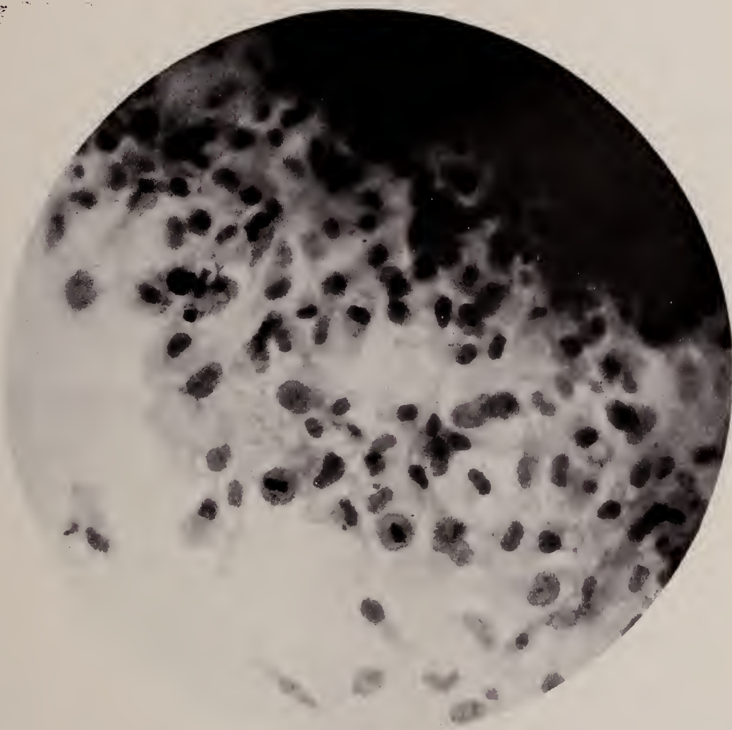


PLATE 9

Fig. 14. Tissue culture, experiment 4, sarcoma, 4 mitotic figures. $\times$ 275.

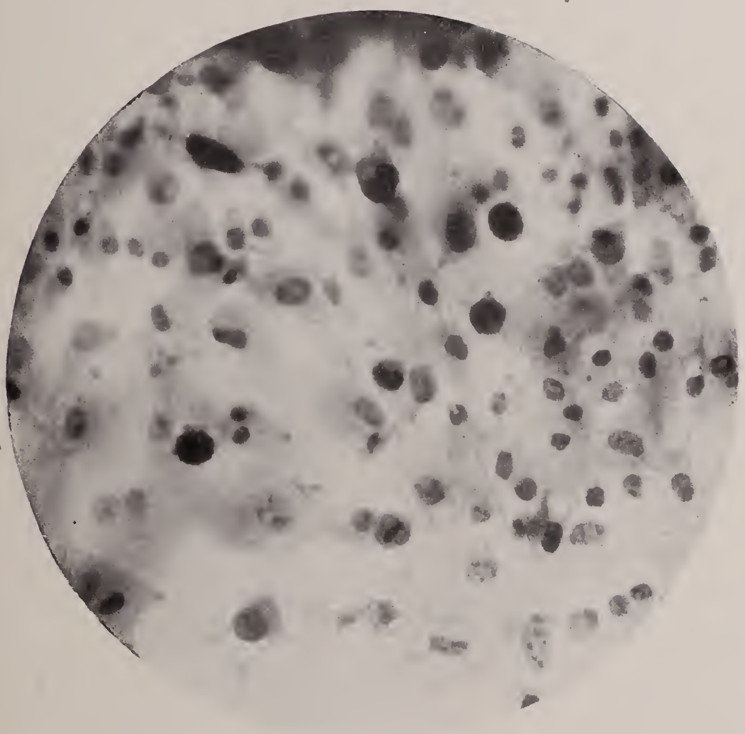


PLATE 10

Fig. 15. Tissue culture, experiment 8, sarcoma, 1 mitotic figure. × 275.

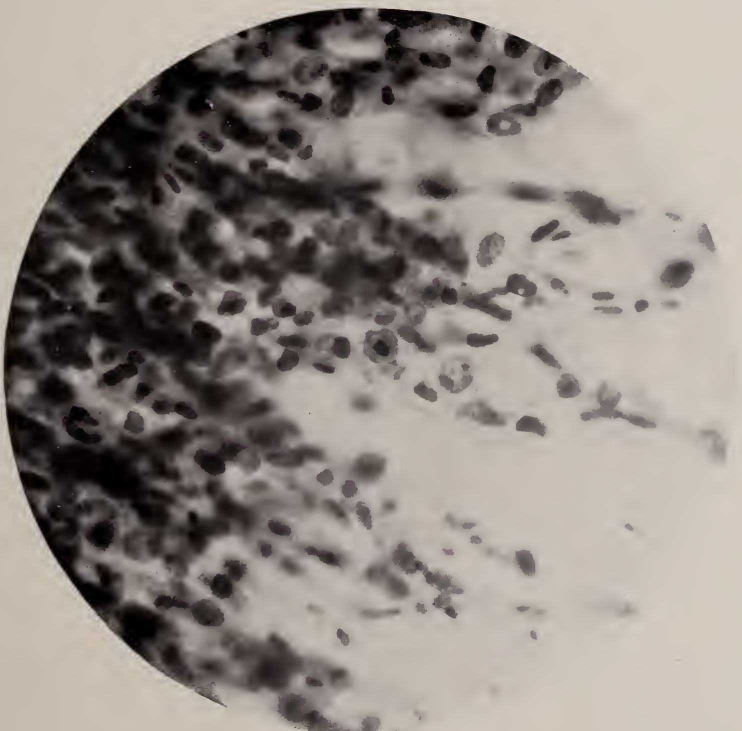


PLATE 11

Fig. 16. Tissue culture, experiment 12, sarcoma, no mitotic figure. × 275.

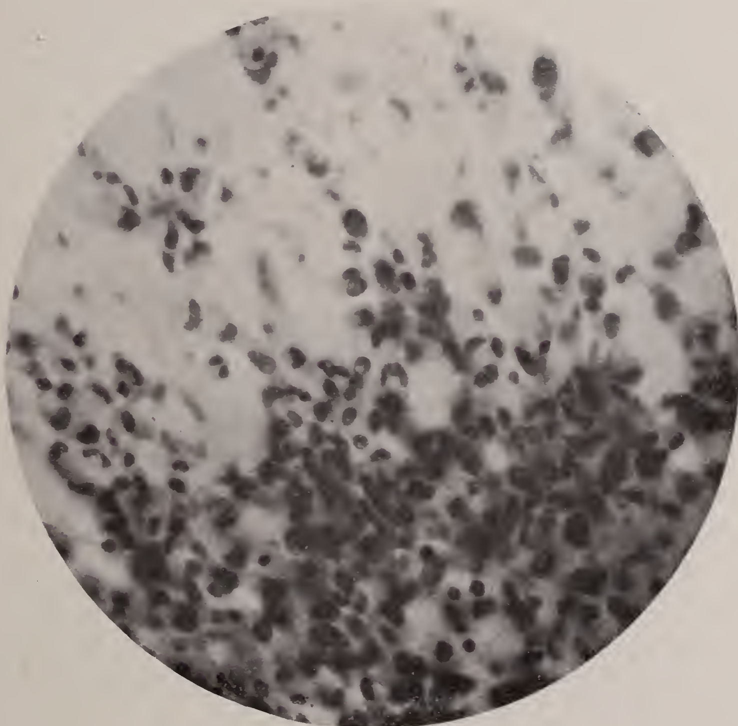


PLATE 12

Fig. 17. Tissue culture, control, sarcoma. $\times$ 37.
Fig. 18. Tissue culture, experiment 4, sarcoma. $\times$ 37.
Fig. 19. Tissue culture, experiment 8, sarcoma. $\times$ 37.
Fig 20. Tissue culture, experiment 12, sarcoma. $\times$ 37.

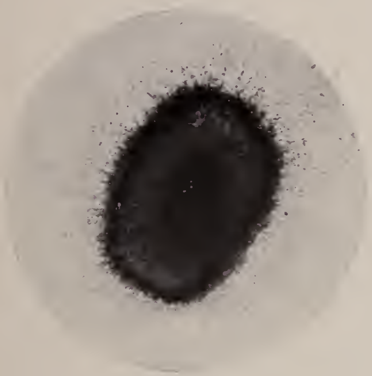


FIG. 17

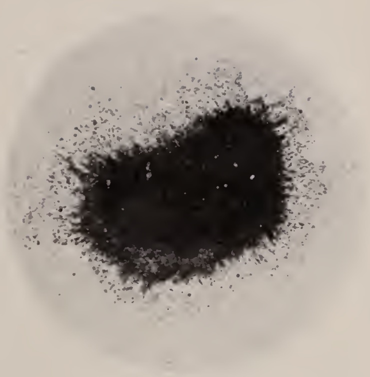


FIG. 18

FIG. 19

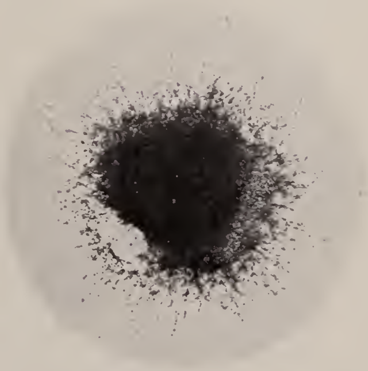


FIG. 20

FURTHER INVESTIGATIONS ON THE ORIGIN OF TUMORS IN MICE

IV. THE TUMOR INCIDENCE IN LATER GENERATIONS OF STRAINS WITH OBSERVED TUMOR RATE.

A. E. C. LATHROP AND LEO LOEB

(From the Department of Comparative Pathology of Washington University School of Medicine, St. Louis.)

Received for publication, January 30, 1918

In previous publications we reported on the difference in the tumor rate which existed in different strains of mice and on the hereditary transmission of this tumor incidence in the simple strains and in the hybrids of different strains. We also gave special attention to the tumor age in the various strains (1). It seemed to us to be of great interest to follow the fate of these strains in order to obtain further insight into the factors which determine the occurrence and origin of tumors in mice. We know that in the course of long continued inbreeding of certain families, without special precautions being taken changes do often occur in the later generations. How far do such changes influence the tumor rate? While our observations are not yet complete, and while further work will be necessary before all the factors involved can be considered satisfactorily analysed, we believe that our present data, which are based on a very extensive material, throw some light on these problems. We shall first consider the various strains, comparing the behavior in the later and in the earlier generations. As to the arrangement of our data and the meaning of the figures, we refer to the explanations given in our earlier papers.

STRAIN LONDON

We have considered in our former paper the F_1, F_2, and F_3 generations of this strain. In those generations the tumor incidence and age were as follows:

Without tumor	*With tumor*
73% (41% I 29% II 30% III)	27% (27% I 43% II 30% III)

The mice belonged to the second age class.

Since then the following groups of *London* mice have been observed:

	Without tumor	*With tumor*
F_3	8 (2 I 1 II 5 III)	
F_4	35 (5 I 13 II 17 III)	15 (4 I 6 II 5 III)
	70% (15% I 37% II 48% III)	30% (26% I 40% II 34% III)
F_5	68 (27 I 23 II 18 III)	25 (12 I 13 II)
	73% (39% I 34% II 27% III)	27% (48% I 52% II)
F_6	39 (12 I 21 II 6 III)	15 (6 I 9 II)
	72% (31% I 54% II 15% III)	28% (40% I 60% II)
Total of F_4, F_5, F_6:		
	142 (44 I 57 II 41 III)	55 (22 I 28 II 5 III)
	72% (31% I 40% II 29% III)	28% (40% I 51% II 9% III)

We see that the tumor incidence in the later generations is almost identical with the incidence of the earlier generations. However, the tumors appear now somewhat earlier than formerly.

In the earlier generations the record of tumor age was as follows:

I Period:	120 mice	9 tumors = 7%	27%	1:11 + III = 1:6.6
II Period:	75 mice	14 tumors = 19%		1:11 = 1:2.7
III Period:	36 mice	10 tumors = 27%		II:III = 1:1.4
				1 + 11:III = 1:1

In the later generations the tumor age was as follows:

I Period:	197 mice	22 tumors = 11%	28%	1: 11 + III = 1: 3
II Period:	131 mice	28 tumors = 21%		I: II = 1: 2
III Period:	46 mice	5 tumors = 11%		II: III = 2: 1
				1 + II: III = 3: 1

The tumors belong now to the I age class, while formerly they belonged to the II age class.

The records of October, 1915, which were intermediate between those of the old and new groups, were as follows:

	Without tumor	*With tumor*
F_3 F_4, F_5	38 (13 I 13 II 12 III)	23 (10 I 12 II 1 III)
	62% (34% I 34% II 32% III)	38% (44% I 52% II 4% III)

The number of mice in this group is somewhat smaller and the tumor incidence is 38 per cent, slightly higher than in the other groups; but the percentage is on the whole of the same order as in the other records. This group, as well as the more recent groups, belongs to the first age class. The tumor rate of the *London* strain as a whole remains constant through the successive generations; this strain nevertheless is a composite of different substrains. Two substrains were detached from the main strain and each substrain was inbred and prevented from interbreeding with other substrains. These substrains differed in tumor rate from the main strain:

Substrains

(a) The offspring of tumor mouse 481, and (b) *London blue and white,* descendants of a group of *London* mice with blue and white color. (a) Family of 481 (offspring of an imported *blue London* mouse in which a tumor developed later.)

1. Records of October, 1915:

	Without tumor	*With tumor*
F_2 and F_3	18 (10 I 5 II 3 III)	0

2. More recent records:

F_3, F_4, F_5	25 (8 I 5 II 12 III)	0

In both groups tumors are entirely lacking through the course of five generations.

(b) *London blue and white:*

F_4 and F_5	14 (10 I 3 II 1 III) 45% (71% I 22% II 7% III)	17 (8 I 7 II 2 III) 55% (47% I 41% II 12% III)

While in this case the tumor rate is higher, this may be accidental and due to the occurrence of a relatively large number of tumors in F_4. Here of 13 mice, 11 had tumors. In the fifth generation the tumor rate was again the one typical for *London* mice, viz., 33 per cent. The tumor age of this group was similar to the one of the other *London* mice; it stood between the first and second age class. The tumor rate of 481 is undoubtedly much lower than the average. It is, therefore, in confirmation of our previous results, possible to split off from larger groups, which were not quite homogeneous, substrains with a different tumor rate.

STRAIN LONDON + (EUROPEAN + 103) F_3

Previously we have considered mainly the F_1 and F_2 generations, with an admixture of a few groups of the F_3 and of one group of the F_4 generation. We have now added to these groups a few mice whose records were written up October, 1915.

The former records were as follows:

Without tumor	*With tumor*
80 (12 I 33 II 35 III)	5 (1 I 2 II 2 III)
95% (15% I 41% II 44% III)	5% (20% I 40% II 40% III)

The tumor age is somewhere between the II and III age class. The new records give mainly the data of the third and fourth generations with some additional data concerning the second and fifth generations.

F_2	5 (2 I 1 II 2 III) 83% (40% I 20% II 40% III)	1 III 7% (100% III)
F_3	20 (3 I 7 II 10 III) 91% (15% I 35% II 50% III)	2 (1 II 1 III) 9% (50% II 50% III)
F_4	26 (8 I 7 II 11 III) 93% (31% I 27% II 42% III)	2 (1 II 1 III) 7% (50% II 50% III)
F_5	7 (1 I 6 III) 100% (14% I 86% III)	

Total of the later generations:

58 (14 I 15 II 29 III)	5 (2 II 3 III)
92% (24% I 26% II 50% III)	8% (40% II 60% III)

The tumor incidence is almost identical in both sets of records. In the individual generations no marked deviation occurs even when the number of mice used is not very great. The tumors appear somewhat later than in the former records; they belong to the fourth age class. However, with such a relatively small number of tumor mice in low tumor strains, a difference in the appearance of one single tumor causes a shifting into another age class. We must, therefore, disregard shifting of age classes in such cases and consider generally whether or not a strain belongs to the earlier or later age classes.

STRAIN HEITLER

In our former paper we gave the records of the first, second, and third generations. We also mentioned a small number of mice of the fourth generation. The records of October, 1915, concern the third, fourth, and fifth generations.

The former records were:

Without tumor	*With tumor*
74 (42 I 18 II 14 III)	28 (4 I 24 II)
73% (56% I 24% II 20% III)	27% (14% I 86% II)

The tumors belong to the second age class. The greater number in the second period is compensated for by the absence of tumors in the III Period.

The new records of October, 1915, are as follows:

Without tumor	*With tumor*
73 (36 I 20 II 17 III)	21 (8 I 9 II 4 III)
77.7% (49% I 27.4% II 23.6% III)	22.3% (38% I 43% II 19% III)

The tumor rates in the earlier and later generations are very similar (27 per cent and 22.3 respectively); also the tumor age is not very different. The tumors stand somewhere between the first and second or third age class, but the tumors appear now somewhat earlier than previously.

1: II + III = 1: 4.4	1: II = 1: 2.1
II: III = 1: 1	1 + II: III = 11.4

STRAIN $8\frac{1}{2}$ + 328 (ENGLISH SABLE)

The older records showed a tumor incidence of 61 per cent. The tumors belonged to the first age class. The records from October, 1915, have been added to the old ones; both combined are as follows:

	Without tumor	*With tumor*
F_1	7 (4 I 3 II)	2 (1 I 1 II)
F_2	17 (12 I 1 II 4 III)	14 (7 I 6 II 1 III)
F_3	12 (8 I 4 II)	32 (22 I 9 II 1 III)
F_4	21 (16 I 5 II)	13 (10 I 3 II)
Total:		
F_1, F_4	57 (40 I 13 II 4 III)	61 (40 I 19 II 2 III)
	49% (70% I 23% II 7% III)	51% ($65\frac{2}{3}$% I 31% II $3\frac{1}{3}$% III)

The newer records are as follows:

	Without tumor	*With tumor*
F_3	35 (16 I 16 II 3 III)	39 (15 I 20 II 4 III)
	47% (46% I 46% II 8% III	53% (39% I 51% II 10% III)
F_4	27 (20 I 4 II 3 III)	15 (10 I 4 II 1 III)
	64% (74% I 15% II 11% III)	36% (67% I 26% II 7% III)
F_5	22 (13 I 8 II 1 III)	44 (25 I 19 II)
	33% (58% I 37% II 5% III)	64% (57% I 43% III)
F_6	7 (3 I 4 II)	8 (3 I 5 II)
	47% (43% I 57% II)	53% (37% I 63% II)
F_7	2 (2 I)	2 (2 I)
	50%	50%

We may add to this record the record of two special families of *8½ + 328,* namely the offspring of tumor mouse 1113 and of tumor mouse 1075 which were *8½ + 328* F_3 July, 1914, groups.

Offspring of no. 1113.

	Without tumor	*With tumor*
F_1, F_2, F_3	2 (2 I)	14 (13 I 1 II)
	12% (100% I)	88% (93% I 7% II)

Offspring of no. 1075:

F_1, F_2, F_3	4 (3 I 1 II)	6 (4 I 1 II)
	40% (75% I 25% II)	60% (67% I 33% II)

If we combine the records of these special families with the newer records of Strain *8½ +328* we obtain the following total.

Without tumor	*With tumor*
99 (59 I 33 II 7 III)	128 (72 I 5 II 5 III)
43% (60% I 33% II 7% III)	57% (56% I 40% II 4% III)

We see, therefore, that in the earlier records (F_1–F_4) the tumor incidence is very similar to the later records (F_3–F_7), namely, 51 per cent as compared to 57 per cent.

We furthermore find that in special families within the larger strain the tumor rate was high and on the whole similar to that

of the main strain. Of course, whenever the number of observed mice becomes relatively small, the variations due to chance are increased.

In another substrain *782a* of (*8½ + 328*) F_2, the records of which are not included in the main strain, the figures are as follows:

a. Former records:

F_1, F_2	7 (5 I 2 II) 32% (71.5% I 28.5% II)	15 (8 I 7 II) 68% (53.5% I 46.5% II)

b. Later records:

F_2, F_3, F_4	21 (15 I 5 II 1 III) 39% (71% I 24% II 5% III)	33 (21 I 12 II) 61% (64% I 36% II)

We see then (1) That the tumor incidence in the later generations is approximately the same as that in earlier generations; and (2) that special families or substrains have the same tumor incidence as the main strain.

The tumor age also remains the same in the later and in the earlier records in the main strain as well as in the individual families.

a. Old records (with the addition of the October, 1915, records):

1: II + III = 1: 2.4	1: II = 1: 1.5
II: III = 1.5: 1	1 + II: III = 2.6: 1

b. New records:

1: II + III = 1: 3	1: II = 1: 1.7
II: III = 1.25: 1	1 + II: III = 2: 1

Both records are very similar; in both the tumors belong to the first age class, or appear even slightly earlier. The same holds good for the substrain *782a*.

Here the old records are:

1: II = 1: 1.7

The new records are:

$$1:\text{II} = 1:1.5$$

In both records all the tumors appear in the first and second periods and the tumors belong to the first age class. Tumor age as well as tumor incidence remain in this case, therefore, constant in the course of successive generations and in substrains or special families.

Substrain 415

No. *415* is a substrain of *101* + (*European* + *103*). No. 415 was a tumor mouse which belonged to the F_2 generation of the main strain. In the main strain the tumor incidence is intermediate between that of the parents, viz., 34 per cent. The tumors belong to the IV age class. In substrain *415* the tumor rate is distinctly lower; it corresponds to that of the mother.

The earlier generations had the following record:

	Without tumor	*With tumor*
F_1, F_2, F_3	16 (8 I 3 II 5 III)	3 (2 I 1 III)
	84% (50% I 19% II 31% III)	16% (67% I 33% III)

There is an intermediate record from October, 1915:

F_3	5 (4 I 1 III)	5 (1 I 4 II)

The later records are as follows:

F_3	21 (6 I 8 II 7 III)	2 (1 II 1 III)
F_4	12 (5 I 6 II 1 III)	4 (1 I 3 II)
F_5	3 (1 I 2 II)	
F_6	18 (16 I 2 II)	1 (1 II)
Total:		
F_3, F_6	54 (28 I 18 II 8 III)	7 (1 I 5 II 1 III)
	89% (52% I 33% II 15% III)	11% (14½% I 71% II 14½% III)

As in the main strain the tumors do not appear early in life. The total of all the generations (F_1–F_6) of 415 is as follows:

Without tumor	*With tumor*
70 (36 I 21 II 13 III)	10 (3 I 5 II 2 III)
87.5% (52% I 30% II 18% III)	12.5% (30% I 50% II 20% III)

The tumor age is as follows:

1: II + III = 1: 7	1: II = 1: 3.2
II: III = 1: 1.1	1 + II: III = 1.2: 1

The tumors belong to the II age class. They appear, therefore, somewhat earlier than in the main strain, which belonged to the fourth age class. We cannot attribute much importance to such differences in the tumor age, for it is liable to occur whenever the number of tumors is relatively small.

STRAIN 121 (ENGLISH + TAN) + CREAM

The former records included the F_1–F_4 generations:

F_1–F_4	53 (20 I 22 II 12 III)	39 (16 I 18 II 5 III)
	58% (37% I 42% II 21% III)	42% (41% I 46% II 13% III)
	II + III 59% III 69%	II + III 41% III 31%

This strain belonged to the II age class.

The intermediate records from October, 1915, are as follows:

F_3 and F_4	49 (23 I 15 II 11 III)	15 (4 I 6 II 5 III)
	76½% (46½% I 31% II 22½% III)	23½ (27% I 39⅔% II 33⅓% III)

The tumor incidence is here lower than in the former records, but the rate is still intermediate between that of the two parents.

The same applies also to the new records:

F_3	9 (3 I 2 II 4 III)	3 (1 I 2 II)
	75% (33⅓% I 22% II 45% III)	25% (33⅓% I 66⅔% II)
F_4	31 (5 I 17 II 9 III)	9 (3 I 4 II 2 III)
	77½% (16% I 55% II 29% III)	22½% (33% I 44% II 23% III)

F_5	31 (5 I 13 II 13 III) 72% (16% I 42% II 42% III)	12 (6 I 4 II 2 III) 28% (51% 33% II 16% III)
F_6	10 (6 I 4 II) 72% (60% I 40% II)	4 (2 I 2 II) 28% (50% I 50% II)
F_7	1 (1 II)	0
Total of the later generations:		
F_3–F_7	82 (19 I 37 II 26 III) 74½% (23% I 45% II 32% III)	28 (12 I 12 II 4 III) 25½% (43% I 43% II 14% III)

The tumor incidence in the later generations is similar to that of the October records and lower than that of the earlier records.

Tumor age of the first generations; II age class:

1: II + III = 1: 8.8	1: II = 1: 4.4
II: III = 1: 1	1 + II: III = 1.2: 1

The tumors of the October, 1915, records stand somewhere between the II and III age class, but are nearer the II age class:

1: II + III = 1: 8	1: II = 1: 2.8
II: III = 1: 1.8	1 + II: III = 1: 1.35

Recent records:

1: II + III = 1: 2.5	I: II = 1: 1.4
II + III = 1.2: 1	1 + II: III = 2.1

The tumors belong now to the first age class; they appear relatively earlier, notwithstanding the decrease in the tumor rate. This is mainly due to the fact that in the later generations the non-tumor mice reach a higher age. Thus the number of tumor mice in the first age period is relatively increased. If we compare the proportion of tumor mice in the various age periods, we find in the later generations almost the same figures as in the earlier ones.

In the earlier generations:

41% I 46% II 13% III

In the later generations:

43% I 43% II 14% III

STRAIN ENGLISH, OCTOBER, 1915, RECORDS

Some groups consisting of *English Sable* and of some other high tumor rate English mice, F_4 and F_7:

Without tumor	*With tumor*
13 (9 I 3 II 1 III)	11 (7 I 3 II 1 III)
54% (70% I 23% II 7% III)	46% (64% I 27% II 9% III)

The tumor rate is still higher although slightly lower than the typical *English* tumor rate; however, the number of mice is relatively small and this might account for the slight variation. The tumors belong to the first age class as is characteristic of the strain *English.*

Substrain English Sable

Former records (F_1–F_5):

76 (49 I 19 II 8 III)	176 (115 I 48 II 13 III)
30% (65% I 25% II 10% III)	70% (66% I 27% II 7% III)

I age class:

1: II + III = 1: 2.8	1: II = 1: 1.5
II: III = 1: 1	1 + II: III = 1.9: 1

New records (F_4–F_7), with exception of family 437.

16 (9 I 4 II 3 III)	32 (13 I 17 II 2 III)
33% (57% I 25% II 18% III)	67% (41% I 53% II 6% III)

The tumor rate remains in the later generations almost the same as in the former ones. The age class remains also approximately the first.

I: II + III = 1:4	1: II = 1: 2.4
II: III = 1.4: 1	1 + II: III = 2.3: 1

While the proportion of the tumors in I: II period is slightly lower, the proportion of the tumors in II: III age class is somewhat higher as compared to the corresponding figures in the earlier generations. The tumor age is slightly higher than formerly; however, the number of mice used is smaller. Among the *English Sable* one separate family, 437 (*English Sable* F_2), was observed which had a slightly higher tumor rate than the average. The former records of 437 with some additional records were as follows:

	Without tumor	*With tumor*
F_1–F_5	5 (5 I)	23 (12 I 11 II)
	18% (100% I)	82% (52% I 48% II)

The tumors belong to the first age class.

The new records of family 437 are as follows:

F_4–F_7 3 (3 I)	24 (20 I 4 II)
11% (100% I)	89% (84% I 16% II)

Here again the high tumor rate is sustained in the later generations and the tumors appear at an early age. We see, then, that within a certain strain a higher tumor rate of a selected family is maintained, and, furthermore, that the tumor age remains the same. In neither of the generations does a tumor appear in the III age period.

There were a few smaller families belonging to substrain *English A* (177a, 881a, 869a 281).

Their tumor rate was:

Without tumor	*With tumor*
17 (13 I 3 II 1 III)	19 (15 I 4 II)
44% (76% I 18% II 6% III)	56% (79% I 21% II)

The high tumor rate is maintained and the tumors belong as usual in this strain to the I age class.

Substrain English Silver

English Silver was a substrain of English that differed markedly from the other substrains; its tumor rate was very low.

The former records were as follows:

Without tumor	With tumor
109 (44 I 49 II 16 III)	8 (4 I 4 II)
93% (41% I 45% II 14% III)	7% (50% I 50% II)

Since then we have obtained a few more records which show the same low tumor rate:

9 (3 I 5 II 1 III)	1 (1 II)
90% (33% I 56% II 11% III)	10% (100% II)

The number of the additional mice is too small for the determination of the tumor age.

Substrain English Silver Fawn

We gave the records of two groups of this strain in our previous paper. It indicated a low tumor rate.

Without tumor	With tumor
13 (6 I 7 II)	0

The later records confirm the conclusion that this substrain differs markedly from the majority of English substrains and approaches the tumor rate of *Silver*, from which they were originally split off and from which they differed only slightly in color.

Without tumor	With tumor
37 (14 I 10 II 13 III)	7 (4 II 3 III)
84% (38% I 27% II 35% III)	16% (57% II 43% III)

The tumor rate is slightly higher than in *Silver*. The tumors appear here distinctly later than in the other English substrains and also later than they did in *English Silver*. Considering the relatively small number of tumor mice observed in the case of the recent groups of *English Silver* and *Silver Fawn*, a determination of the age class of these tumor mice can be made only with reservation.

STRAIN CREAM

This is a strain differing markedly from the English in tumor rate and tumor age. The tumor rate is low and the tumors appear late.

The former records of *Cream* were as follows:

Without tumor	*With tumor*
221 (63 I 91 II 67 III)	5 (2 II 3 III)
98% (29% I 41% II 30% III)	2% (40% II 60% III)
II + III 97% III 96%	II + III 3% III 4%

This record does not include certain substrains like *Cream X* and *Cream G*.

The new records of the later generations are as follows:

Cream A (mostly black color):

Without tumor	*With tumor*
105 (32 I 41 II 32 III)	9 (2 I 3 II 4 III)
92% (30.5% I 39% II 30.5% III)	8% (22% I 33% II 45% III)

The tumor rate is very low and the tumors appear late.

Cream B (mostly white color):

Without tumor	*With tumor*
72 (24 I 21 II 27 III)	17 (1 I 9 II 7 III)
87% (33% I 28% II 39% III)	19% (6% I 53% II 41% III)

There is included in this substrain a group with a somewhat higher tumor rate. These mice are the offspring of a family which was very prolific (June, 1914, family).

This group had the following tumor rate:

Without tumor	*With tumor*
26 (6 I 6 II 14 III)	14 (1 I 6 II 7 III)
65% (23% I 23% II 54% III)	35% (7% I 43% 50% III)

While the tumor rate is here higher, the tumors appear late. The rest of this substrain, *Cream B*, with the exclusion of the June, 1914, family, has the typical low tumor rate.

Without tumor	*With tumor*
46 (18 I 15 II 13 III)	3 (3 II)
94% (39% I 33% II 28% III)	6% (100% II)

We may, therefore, conclude that no fargoing change has taken place in the *Cream* strain as a whole in regard to tumor incidence or tumor age, and that the change which affects apparently the whole strain is due to the prevalence within the strain of a certain family with a tumor rate that is higher than the average.

Substrain Cream Black (offspring of the original Cream, but mostly with black color)

a. Intermediate records of October, 1915.

Without tumor	*With tumor*
74 (26 I 27 II 21 III)	3 (1I 2 III)
96% (35.5% I 36.5% II 28% III)	4% ($33\frac{1}{3}$% I $66\frac{2}{3}$% III)

b. The newer records of *Cream Black* are as follows:

Without tumor	*With tumor*
87 (31 I 25 II 31 III)	11 (3 I 2 II 6 III)
89% (35% I 30% II 35% III)	11% (27% I 19% II 54% III)

Although in the newer records of this substrain the tumor rate is still very low, it is somewhat higher than in the intermediate records and in *Cream* as a whole. The tumors appear late in life as usual in *Cream* strain.

Substrain Cream X

a. Old records:

Without tumor	*With tumor*
130 (48 I 45 II 37 III)	5 (2 I 3 II)
96% (37% I 35% II 28% III)	4% (40% I 60% II)

b. Intermediate records of October, 1915.

Without tumor	*With tumor*
75 (26 I 26 II 23 III)	2 (1 II 1 III)
97.4% (34.5% I 34.5% II 31% III)	2.6% (50% II 50% III)

c. New records:

Without tumor	*With tumor*
48 (13 I 20 II 15 III)	0
100% (27% I 42% II 31% III)	0%

The total of the new records (excluding records of October, 1915) of these groups of *Cream* including *Cream X* is as follows:

Without tumor	*With tumor*
312 (100 I 107 II 105 III)	37 (6 I 14 II 17 III)
89½ (32% I 35% II 33% III)	10½% (16% I 37% II 47% III)

While the tumor rate is still very low, it is somewhat higher than previously, and the tumors appear now at a slightly earlier period in life. The total of the new records of the corresponding substrains was as follows:

Without tumor	*With tumor*
98% (29% I 41% II 30% III)	2% (40% II 60% III)

There is one special family of *Black Cream* which was detached from the main strain for special reasons and called *Cream Y*. In this family the tumor rate was as follows:

Without tumor	*With tumor*
14 (6 I 5 II 3 III)	0
100% (43.5% I 35% II 21.5% III)	0%

Here tumors were absent.

Tumor age of the *Creams* is as follows:

a. The tumor age in the old *Cream* records (with exclusion of *Cream X* and *Y*.)

1: II + III = 1: 00	1: II = 1: 00
II: III = 1: 3.6	1 + II: III = 1: 3.6

The tumors appear later than in the IV age class.

In the new *Cream* records the tumor age is as follows:

b. Cream A:

1: II + III = 1: 8.7	1: II = 1: 2.1
II: III = 1: 3	I + II: III = 1: 2

This corresponds to the IV age class as far as the relation of II: III, and to the II age class as far as the relation of the I: II period is concerned.

c. Cream B:

1: II + III = 1: 32	1: II = 1: 13
II: III = 1: 1.5	1 + II: III = 1: 1.4

This corresponds to the IV age class.

d. The offspring of the prolific group among *Cream B:*

1: II + III = 1: 20	1: II = 1: 7
II: III = 1: 1.8	1 + II: III = 1: 1.6

Notwithstanding the higher tumor rate in this group, the tumors belong likewise to the IV age class.

e. Cream Black:

1: II + III = 1: 6.7	1: II = 1: 1
II: III = 1: 5.5	1 + II: III = 1: 2.8

The relation of the I to the II period places these tumors in the II age class, but as far as the relation of the II: III period is concerned, they belong to the IV age class.

f. The former *Cream X* records:

1: II = 1: 2.3	1: II + III
II: III = 3.50	1 + II: III = 5: 0

The tumors belong to the I age class.

g. Total of all new *Cream*, including *Cream X*:

1: II + III = 1: 7	I: II = 1: 3.4
II: III = 1: 1.2	1 + II: III = 1: 1

The tumors belong to the II age class.

The tumors belong to the late age classes, and the record is maintained in later generations.

STRAIN NO. 8

The former figures for this strain are as follows:

	Without tumor	*With tumor*
F_2–F_{10}	149 (70 I 33 II 37 III) 9 unknown age	64 (17 I 24 II 23 III)
	70% (47% I 22% II 25% III)	30% (29% I 37% II 34% III)
	II + III 60% 111 63%	II + III 38% III 37%

The intermediate October, 1915, and new records comprise F_{12}, F_{13}, F_{14}.

Without tumor	*With tumor*
27 (6 I 9 II 12 III)	3 (3 III)
90% (22.5% I 35% II 44.5% III)	10% (100% III)

In this group there was a decrease in the tumor rate which became noticeable as early as the F_7 generation. If we divide the old records into two groups, the first group comprising F_2–F_6 and the second F_7–F_{10}, we find a marked difference.

	Without tumor	*With tumor*
.I Group	96 (44 I 22 II 30 III)	49 (17 I 18 II 14 III)
F_2–F_6	66% (46% I 23% II 31% III)	34% (35% I 38% II 28% III)
II Group	53 (29 I 14 II 10 III)	15 (6 II 9 III)
F_7–F_{10}	78% (55% I 26% II 19% III)	22% (40% II 60% III)

We notice, then, in this strain, a gradual decrease in the tumor rate in the later generations.

With tumors: F_2–F_6 34% F_7–F_{10} 22% F_{12}–F_{14} 10%

At the same time the tumors appear correspondingly later in life. The first generations belong to the II, the later ones to the IV age class. At first approximately one-third of the tumors appear in the first period, in the second group none appear in the first period, but four-tenths appear in the second period, while at last all the tumors appear in the III period of life. Parallel with this decrease in the tumor rate and the increase in tumor age, we find a decrease in the fertility of this strain.

STRAIN $8\frac{1}{2}$

In the former records (F_1–F_5), with the addition of a few F_6 mice, the figures were as follows:

Without tumor	*With tumor*
131 (43 I 52 II 36 III)	27 (6 I 17 II 4 III)
83% (33% I 40% II 27% III)	17% (22% I 63% II 15% III)
II + III 81% III 90%	II + III 19% III 10%

Intermediate records from October, 1915:

	Without tumor	*With tumor*
F_4–F_6	28 (15 I 8 II 5 III)	0
	100% (54% I 28% II 18% III)	0%

New records F_6–F_8:

Without tumor	*With tumor*
50 (20 I 25 II 5 III)	5 (1 I 3 II 1 III)
91% (40% I 50% II 10% III)	9% (20% I 60% II 20% III)

The tumor frequency was low from the beginning. A decrease took place in the later generations, somewhat as in strain *no. 8*. The tumors also appear now somewhat later than previously.

1: II + III = 1: 13	1: II = 1: 4.5
II + III = 1: 1.9	1 + II: III = 1: 1.5

In the later generations they stood between the III and IV age class, while formerly they belonged to the II age class.

STRAIN EUROPEAN

In our previous communications we distinguished two groups, the ordinary *European* and the descendants of the *Trio*. The figures for the sum of both were as follows:

	Without tumor	*With tumor*
F_2–F_5	95 (37 I 33 II 25 III)	18 (7 I 8 II 3 III)
	84% (39% I 34% II 27% III)	16% (39% I 44% II 17% III)

Intermediate records of October, 1915; we again give the combined figures, the ordinary *European* and the descendants of the *Trio*.

	Without tumor	*With tumor*
F_4, F_5, F_6	79 (28 I 33 II 18 III)	3 (2 I 1 II)
	96.4% (35.5% I 42% II 22.5% III)	3.6% (67% I 33% II)

The new records are as follows:

	Without tumor	*With tumor*
F_5–F_8	66 (24 I 22 II 20 III)	2 (2 III)
	97% (37% I 33% II 30% III)	3% (100% III)

If we combine the intermediate and the new records, we obtain the following figures:

Without tumor	*With tumor*
145 (52 I 55 II 38 III)	5 (2 I 1 II 2 III)
96.7% (36% I 37% II 27% III)	3.3% (40% I 20% II 40% III)

We see, then, that while strain *European* does not essentially change its character, remaining poor in tumors, the tumor rate decreases from 16 per cent to 3.3 per cent. Formerly the tumors stood between the I and II age class as far as the relations between I and II age period are concerned, and they belonged to the I age class as through the relations between the II and III periods. Now the tumors belong partly to the I

age class (I: II age period) and partly to the III–IV age class (II: III age periods). In determining the age class, we must remember that if the number of tumors in a strain is very small, a few tumor mice may accidentally change the age class of the strain. This is liable to occur in the case of strains with a very low tumor rate.

HYBRIDS (EUROPEAN +102 OR 103)

Former tumor rate of *European +102*, 14 per cent (17 per cent). The tumors belonged to the IV age class.

European +103 had formerly a tumor rate of 10 per cent.

The tumor rate of the total of these hybrids (including some mixed families) is 21 per cent:

Without tumor	*With tumor*
116 (35 I 24 II 57 III)	30 (2 I 9II 19 III)
79.5% (30% I 21% II 49% III)	20.5% (7% I 30% II 63% III)

The October, 1915, records of *European +102* were as follows:

	Without tumor	*With tumor*
F_5	21 (18 I 3 II)	I (1 II)

The new records of *European +102*:

	Without tumor	*With tumor*
F_4, F	44 (19 I 15 II 10 III)	1 (1 III)
	97.8% (43% I 34% II 23% III)	2.2% (100% III)

The new records of *European +103*:

	Without tumor	*With tumor*
F_1–F_3	6 (1 I 1 II 4 III)	2 (1 II 1 III)
	75% (16½% I 16½% II 67% III)	25% (50% II 50% III)

Total:

Without tumor	*With tumor*
71 (38 I 19 II 14 III)	4 (2 II 2 III)
94.7% (53% I 27% II 20% III)	5.3 (50% II 50% III)

As formerly, the tumor rate is very low in the newer records, but the tumor rate is lower now than it was formerly. Again the tumors belong to the IV age class.

Hybrids (European +8 F_5)

Former records:

	Without tumor	*With tumor*
F_1–F_4	88 (31 I 19 II 38 III) 70% (35% I 22% II 43% III)	37 (8 I 20 II 9 III) 30% (22% I 54% II 24% III)

Combined records of October, 1915, and new records:

	Without tumor	*With tumor*
F_3–F_6	20 (7 I 5 II 8 III) 77% (35% I 25% II 40% III)	6 (1 I 2 II 3 III) 23% (17% I 33% II 50% III)

The tumors belonged formerly to the II age class; they now stand between the II and III age class. The new records are therefore very similar to the old ones. The tumor rate is now slightly lower and the tumors appear only a little later than formerly.

hybrids (european +102) F_1+8½ F_4

Former records:

	Without tumor	*With tumor*
F_1–F_5	396 (101 I 112 II 183 III) 84% (25% I 28% II 47% III)	77 (8 I 19 II 50 III) 16% (10% I 25% II 65% III)

The tumors belong to the IV age class.
Records of October, 1915.

F_2–F_5	59 (17 I 15 II 27 III) 87% (29% I 25% II 46% III	9 (2 I 4 II 3 III) 13% (22% I 44⅔% II 33⅓% III)

The tumors stand between the II and III age classes.

New records:

	Without tumor	*With tumor*
F_3–F_7	50 (15 I 12 II 23 III)	11 (1 I 3 II 7 III)
	82% (30% I 24% II 46% III)	18% (9% I 27% II 64% III)

The tumor rate is approximately unchanged.

The tumor age of the new records also is similar to the former records:

1: II + III = 1: 19	1: II = 1: 5
II: III = 1: 3.3	I + II: III = 1: 2.4

The tumors belong again to the IV age class.

HYBRIDS (EUROPEAN 151+II DAUGHTER OF NO. 10 (NOVEMBER 8TH STRAIN)

Former records:

F_1–F_4	34 (15 I 16 II 3 III)	62 (20 I 33 II 9 III)
	35% (44% I 47% II 9% III)	65% (32% I 54% II 14% III)
	II + III 31%	II + III 69% II Age class

Records of October, 1915 and new records combined (including the records of family 593):

F_4–F_7	51 (32 I 12 II 7 III)	40 (17 I 15 II 8 III)
	56% (63% I 23% II 14% III)	44% (43% I 37% II 20% III)
	II + III 45%	II + III 55%

The tumor rate of this strain remains high; it is, however, not quite so high as before. This is in part due to the great number of deaths in the first age class among the non-tumor animals in the new records. In these "II+III" shows, therefore, a somewhat higher tumor rate. The age class of this tumor

is approximately the II class. It belonged formerly, also, to the II age class.

1: II + III = 1: 5	1: II = 1: 2
II: III = 1: 1.5	1 + II: III = 1: 1.2

HYBRIDS ($8\frac{1}{2}$ + II DAUGHTER OF NO. 10)

Former records:

	Without tumor	*With tumor*
F_1–F_4	42 (8 I 14 II 20 III)	40 (6 I 19 II 15 III)
	51% (19% I 33% II 48% III)	49% (15% I 47½% II 37½% III)
II + III	50%	II + III 50%

New records:

	Without tumor	*With tumor*
F_4–F_6	24 (8 I 9 II 7 III)	4 (4 II)
	86% (33% I 38% II 29% III)	14% (100% II)
	II + III 80%	II + III 20%

There is a noticeable decrease in the tumor rate which is probably not an accident due to the small number of mice in the new series. As before the tumors appear late.

STRAIN (EUROPEAN +103) F_1 + III DAUGHTER OF NO. 10

Former records:

	Without tumor	*With tumor*
F_1–F_3	140 (50 I 37 II 53 III)	28 (3 I 11 II 14 III)
	83% (36% I 27% II 37% III)	17% (11% I 39% II 50% III)

These tumors belong to the IV age class.
Records of October, 1915:

	Without tumor	*With tumor*
F_3, F_4	26 (13 I 13 II)	4 (2 II 2 III)
	87% (50% I 50% II)	13% (50% II 50% III)

Here again the tumors belong to the IV age class.

New records:

	Without tumor	*With tumor*
F_3, F_4, F_5	21 (7 I 7 II 7 III)	0
	100% (33⅓% I 33⅓% II 33⅓% III)	0%

The tumor rate remains throughout low, and tumors appear late, but a decrease apparently takes place in the later generations.

STRAIN (EUROPEAN (151) + I DAUGHTER OF NO. 10 (NOVEMBER 3RD) + 101 ENGLISH)

Former records:

	Without tumor	*With tumor*
F_1–F_4	12 (8 I 2 II 2 III)	15 (10 I 5 II)
	45% (66% I 17% II 17% III)	55% (66⅔% I 33⅓% II) I Age class.

October, 1915, records:

F_3–F_4	14 (9 I 5 II)	8 (3 I 3 II 2 III)
	64% (64% I 36% II)	36% (37.5% I 34.5% II 25% III)

New records:

	Without tumor	*With tumor*
F_4–F_6	10 (7 I 2 II 1 III)	3 (3 I)
	77% (70% I 20% II 10% III)	23% (100% I)

Age of death of non-tumor mice, intermediate and new records combined:

24 (16 I 7 II 1 III) = 66.6% I 29.2% II 4.2% III

While the number of mice in the new records is not large, still it is probable that an actual decrease in the tumor rate has taken place in this strain.

Tumor age of intermediate and new groups combined:

1:II + III = 1:5	1:II = 1:1.4
II:III = 1:3	1 + II:III = 1.1.7

They stand between I and II age classes in part, and in part they belong to III age class.

HYBRIDS (EUROPEAN + ENGLISH TAN (DAUGHTER OF TUMOR MOUSE 146)).

Former records:

	Without tumor	*With tumor*
F_1–F_3	53 (13 I 25 II 17 III) 72% (24% I 43% II 33% III)	21 (3 I 13 II 5 III) 28% (14% I 62% II 24% III)

II age class.

In this case F_3 had a very low tumor rate.
F_1–F_2 gave a higher record:

23 (5 I 12 II 6 III) 56% (22% I 52% II 26% III)	18 (3 I 11 II 4 III) 44% (16% I 61% II 23% III)

The new records:

F_4–F_6	19 (3 I 10 II 6 III) 58% (16% I 52% II 32% III)	14 (1 I 11 II 2 III) 42% (7% I 79% II 14% III)

The new records are very similar to the F_1 and F_2 generations of the former records. But the F_3 generation of the former record had a very low tumor incidence.

Tumor age:

Formerly in I period 4%	now in I period 3%
Formerly in .II period 22%	now in II period 38%
Formerly in III period 23%	now in III period 25%

= II class.

= II class.

I: II + III = 1: 21	I: II = 1: 13
II: III = 1.5: 1	I + II: III = 1.6: I

I: II = IV age class
II: III = I age class.

The large majority of the tumor mice die in this strain in the II age period.

HYBRIDS (SILVER + 10)

Former records:

Without tumor	*With tumor*
150 (54 I 50 II 46 III)	84 (26 I 29 II 29 III)
64% ($35\frac{2}{3}$% I $33\frac{1}{3}$% II 31% III)	36% (32% I 34% II 34% III)

II age class.

New records:

Without tumor	*With tumor*
4 (2 I 2 II)	7 (3 I 2 II 2 III)
36% (50% I 50% II)	64% (43% I 28.5% II 28.5% III)

While the number of mice is too small to allow an exact comparison with the tumor rate of the former records, still the record is of value as indicating that the low tumor rate of the *Silver* was not dominant over the *no. 10* strain and that the tumor rate was maintained fairly high.

HYBRIDS (CREAM + I DAUGHTER OF NO. 10)

Former records:

F_1–F_4	112 (18 I 33 II 61 III)	62 (4 I 23 II 35 III)
	64% (16% I 29% II 55% III)	36% (6% I 37% II 57% III)

IV age class.

New records:

F_4–F_6	35 (10 I 11 II 14 III) 74% (28% I 31% II 41% III)	12 (4 I 6 II 2 III) 26% (33% I 50% II 17% III)

Age class:

I period:	8.5%	1: II + III = 1:3.7	1: II = 1:2.2
II period:	19%	II: III = 1.5:1	1 + II: III = 2.2:1
III period:	12.5%		

The tumor belongs to the I age class.

While the tumor rate is somewhat lower than previously, no essential change has taken place in this respect. The tumors appear earlier than previously; however, considering the relatively small number of tumors observed in the later generations no great importance can be attached to this change.

HYBRIDS (MICHIGAN WILD + ENGLISH 101)

Former records:

F_1–F_4	21 (14 I 4 II 3 III) 42% (67% I 19% II 14% III)	29 (13 I 9 II 7 III) 58% (45% I 31% II 24% III)

I age class

New records (including October, 1915, records):

F_3, F_5, F_6	15 (8 I 4 II 3 III) 75% (53% I 27% II 20% III)	5 (3 I 1 II 1 III) 25% (60% I 20% II 20% III)	
I Period:	15%	1: II + III = 1:2.4	1: II = 1.3:1
II Period:	11%	II: III = 1:2.3	I + II: III = 1:1
III Period:	25%		

The tumor age is partly I class, partly II and III classes. Here again a decrease in the tumor rate is noticeable. The tumor age remains essentially unchanged.

HYBRIDS (ENGLISH TAN AND GERMAN)

Former records:

Without tumor	*With tumor*
25 (11 I 7 II 2 III)	19 (10 I 7 II 2 III)
51% (55% I 35% II 10% III)	49% (52% I 36% II 12% III)

I age class

New records:

	Without tumor	*With tumor*
F_3–F_7	12 (4 I 6 II 2 III)	9 (4 I 5 II)
	57% (33% I 50% II 17% III)	43% (44½% I 55½% II)

I age class

The new records are very similar to the old ones. We cannot be certain about the character of this strain but we had reason to believe that it belongs to the class of English mice. The records bear out this suggestion, inasmuch as the tumor rate was high and the tumors appeared early, just as in the case of the *English* strain.

Former records:

	Without tumor	*With tumor*
F_1–F_5	7 (3 I 3 II 1 III)	15 (6 I 8 II 1 III)

New records:

F_4–F_6	2 (2 II)	8 (8 I)

While the number of mice observed is not great, the new records confirm the older ones.

Total:

Without tumor	*With tumor*
9 (3 I 5 II 1 III)	23 (14 I 8 II 1 III)
28% (33% I 56% II 11% III)	72% (60½% I 35% II 4½% III)

I Period:	44%	1: II: III = 1: 2.3	1: II = 1: 1.2
II Period:	53%	II: III = 1: 1	1 + II: III = 2:1
III Period:	50%		

I age class

STRAIN GERMAN

At no time did we have a large number of mice in this strain; however, all indications point to the conclusion that the tumor rate in this strain is fairly high.

Former records:

Without tumor	*With tumor*
10 (6 I 3 II 1 III)	10 (3 I 5 II 2 III)
50% (60% I 30% II 10% III)	50% (30% I 50% II 20% III)

II age class

Records of October, 1915:

	Without tumor	*With tumor*
F_4–F_6	11 (7 I 4 II)	5 (3 I 2 II)
	69% (64% I 36% II)	31% (60% I 40% II)

New records:

F_7	4 (2 I 2 II)	2 (2 I)
	66⅔% (50% I 50% II)	33⅓% (100% I)

The tumor rate is somewhat lower than it was previously. The tumors appear early. Formerly the tumors belonged to the II age class. The small number of tumor mice in the later records makes the determination of the age class uncertain.

HYBRIDS (8 + GERMAN)

Former records:

	Without tumor	*With tumor*
F_2–F_5	144 (40 I 46 II 58 III)	100 (21 I 51 II 28 III)
		41% (21% I 51% II 28% III)

(Including a few mice from later generation.)
59% (28% I 32% II 40% III)
II + III 59% II + III 41%
II age class

Records of October, 1915:

F_3, F_4, F_6	27 (1 I 21 II 5 III)	8 (5 I 3 II)
	77% (3.7% I 77.8% II 18.5% III)	23% (62.3% I 37.5% II)

New records:

F_4–F_9	75 (22 I 24 II 29 III)	19 (4I 12 II 3 III)
	80% (30% I 32% II 38% III)	20% (21% I 63% II 16% III)

There is in this strain a noteworthy fall in the tumor rate, which was already apparent in the intermediate and was maintained in the new records:

New records:

I Period:	4.2%	1: II + III = 1: 6.5	1: II = 1: 4.3
II Period:	18%	II : III = 2: 1	1 + II: III = 2.5: 1
III Period:	9%		

The tumors stand between I and II age class, but are nearer the II age class.

Included among the *8 + German* in our previous report appeared a family which was the offspring of tumor mouse no. 240. This family we used for further hybridization and we therefore give this record:

NO. 240 F–F_3 = 8 + GERMAN F_4–F_7

Without tumor	*With tumor*
8 (4 I 3 II 1 III)	6 (4 I 2 II)
57% (50% I 37½% II 12½% III)	43% (67% I 33% II)

The tumor rate is here very similar to that of *8 + German*.

198 (ENGLISH) + (8+GERMAN) F_4

Former records:

Without tumor	*With tumor*
30 (22 I 7 II 1 III)	52 (27 I 25 II)
37% (74% I 23% II 3% III)	63% (52% I 48% II)
II + III 24%	II + III 76%

I age class

Records of October, 1915:

	Without tumor	*With tumor*
F_2, F_3	8 (3 I 5 II)	20 (11 I 8 II 1 III)
	29% (37% I 63% II)	71% (55% I 40% II 5% III)
	1: II + III = 1: 4	1: II = 1: 1.4
	II: III = 1: 1.75	1 + II: III = 1: 1

Between I and II age class

New records:

	Without tumor	*With tumor*
F_2–F_6	61 (42 I 16 II 3 III)	46 (23 I 19 II 4 III)
	57% (69% I 26% II 5% III)	43% (50% I 41% II 9% III)
	II + III 45%	II + III 55%

I Period: 22.5%	1: II + III = 1: 4.5	1: II = 1: 2
II Period: 45%	II: III = 1: 1.3	1 + II: III = 1.2: 1.
III Period: 57%		

Between I and II age class

The tumor rate remains high, and higher in II + III than in the sum of all age classes, just as in the earlier records. On the whole, however, a decrease has taken place in the tumor rate from 63% to 43%. The tumor age has increased only slightly.

HYBRIDS (GERMAN + CARTER)

Former records:

	Without tumor	*With tumor*
F_1–F_4	326 (79 I 88 II 159 III)	32 (4 I 11 II 17 III)
	91% (24% I 27% II 49% III)	9% (12½% I 39% II 48½% III)

IV age class

I Period: 1%	1: II + III = 1: 14	1: II = 1: 4
II Period: 4%	II: III = 1: 2.5	1 + II: III = 1: 2
III Period: 10%		

New records:

	Without tumor	*With tumor*
F_4–F_6	75 (32 I 22 II 21 III) 66½% (43% I 29% II 28% III)	38 (16 I 13 II 9 III) 33½% (43% I 34% II 23% III)

I Period: 14%	1: II + III = 1: 3.5	1: II = 1: 1.4
II Period: 20%	II: III = 1: 1.5	1 + II: III = 1 :1
III Period: 30%		

The records of this strain had in all probability been abnormal, inasmuch as the tumor rate of the hybrids was much lower than that of either parent. Now the tumor rate has increased and has become similar to that of *Carter*. At the same time the tumors appear earlier.

One family of strain *German + Carter*, the offspring of tumor mouse No. 794 (= *German + Carter F_3*), was kept separate.

The records of No. 794 are as follows:

794 F_1–F_4 = GERMAN + CARTER F_4–F_7

Without tumor	*With tumor*
16 (8 I 5 II 3 III) 64% (50% I 31% II 19% III)	9 (1 I 4 II 4 III) 36% (11% I 44½% II 44½% III)

..I Period: 4%	1: II + III = 1: 20.5	1: II = 1: 6
.II Period: 24%	II: III = 1: 2.3	1 + II: III = 1:1.9
III Period: 57%		

Tumors appear here late as they did in the early *German + Carter* records. They belong almost to the IV age class.

DISCUSSION

From an analysis of our records, we may conclude that a considerable majority of our tumor strains, viz., twenty strains or substrains, maintained a tumor rate approximately unchanged throughout the period of observation. On table 1 these strains are recorded. On the whole, the constancy of the percentages is surprising in most of the cases. In a few strains there is noticeable a very slight rise, in others a slight decrease in the tumor rate; the latter preponderates over the rise. In one case (*Cream + 10*) the descent is somewhat more marked (from 36 per cent to 26 per cent), so that it might be queried whether this strain would not, perhaps, have been more suitably placed among those showing a decline in the tumor rate. The strains in which the tumor rate is approximately constant comprise all kinds—high, as well as medium and low tumor rate strains.

There are only two strains in which the tumor rate has risen (table 2). Such an increase was found in some of the *Cream* substrains and in the *German* and *Carter* strain. While *Cream X* remained unchanged, on the whole, or fell slightly, we find a small increase in the case of *Cream A*; and among the *Cream* mice a substrain, *Cream B*, was developed, which had a noticeably higher tumor rate. The rate of *Cream B* rose to 19 per cent, while the average of *Cream* as a whole had been below 10 per cent. If we analyze still further the increase in the tumor rate of *Cream B*, we find that it affects principally one family (June, 1914, family); here the rate rose as high as 35 per cent, while in the rest of *Cream B* it remained typically low, namely, 6 per cent. We may, therefore, conclude that the rise in the tumor rate is due to the fact that certain families, which had from the beginning a potentially higher rate, but were at first not numerically important, gained in the course of time a certain numerical preponderance over the other substrains and thus caused apparently an increase in the tumor rate of the whole strain. This is an occurrence comparable to an apparent increase in the number of takes in transplanted tumors, which we have observed in certain strains in the case of continued trans-

TABLE 1

Unchanged or slightly decreasing tumor rate

TUMOR RATE			STRAIN	TUMOR AGE		
Previous	Intermediate	Recent		Previous	Intermediate	Recent
per cent	*per cent*	*per cent*				
27	38	28	*London*	II	I	I
		0	*481 London*			
		55	*London blue white*			I–II
5		8	*London + (European + 103)* F_3	II–III		IV
27	22.3		*Heitler*	III–I	I–II	
51		57 (Total)	$8\frac{1}{2} + 328$	I		I
		88	*1113 =* $(8\frac{1}{2} + 328)$			
		60	*1075 =* $(8\frac{1}{2} + 328)$			
68		61	*782a =* $(8\frac{1}{2} + 328)\ F_2$	I		I
16		11	*415 = 101 + (Europe + 103)*	Between I and III		II
12.5			Total 415 ($F_1 - F_6$)	II		
34			*Main strain 101 + (Europe + 103)*	IV		
		46	*English*			I
70		67	*English Sable*	I		1 (II)
82		89	*437 (English Sable)*	I		I
63		56	*English A*	I		I
7		10	*English Silver*	I		
Very low		16	*Silver Fawn*			IV
4	2.6	0	*Cream X*	I		IV
0			*Cream Y*	IV		
30	23		*European +* $8F_5$	II		II–III
16	13	18	*(European + 102) +* $8\frac{1}{2}\ F_4$	IV	II-III	IV
$F_1 - F_2$, 44	$F_1 - F_3$ 28	42	*European + English Tan (146)*	II		IV–I
36		26	*Cream + 10*	IV		I
49		43	*English Tan + German*	I		I
High		High	Uncertain	I		
8 + German 41		43	*No. 240 = 8 + German*	II		I

plantation (2). In this case we could show that an apparent change in the susceptibility to tumor inoculation of a certain strain was only apparent, being due to the gradual numerical preponderance of a certain family within the strain. A certain family propagates more rapidly, either as a result of accident, or in consequence of a greater resistance to certain diseases, or as a result of a naturally greater prolificness. Another undoubted rise in the tumor rate was observed in the case of *German + Carter;* where formerly the tumor rate had been but 9 per cent, the new records show a rate of 33 to 36 per cent. The former tumor rate in this case had been abnormally low, lower than that of either of the parents, but in the later records it approaches that of the mother strain. We should, therefore, have to explain the former low rate, rather than the later rise. This we are not able to do at present, but we suspect that here again the preponderance in earlier generations of a certain family with a low tumor rate was responsible for the very low

TABLE 2

Increasing tumor rate

TUMOR RATE			STRAIN	TUMOR AGE		
Previous	Intermediate	Recent		Previous	Intermediate	Recent
per cent	*per cent*	*per cent*				
2 (Total)		8	*Cream A*	IV* (Total)		II–IV
		19	*Cream B* total			IV
		35	*Cream B* June 1914 family			IV
		6	*Cream B* without June 1914 family			
	4	11	*Cream Black*			II–IV
2		10½	Total *Creams* including *Cream X*	IV		II
36		64 ? small number	*Silver + 10*	II		
9		33½	*German + Carter*	IV		Almost I
		36	*No. 79¼-German + Carter*			Almost IV

* Total with exclusion of *Creaam X* and *Y*.

rate of the whole strain. The rise in *Silver + 10* may be without significance, considering the very small number of animals which composed the later generations.

Very much more considerable is the number of strains in which the tumor rate has decreased in the later generations (table 3). Thirteen strains show this fall, though quantitatively the decrease varies considerably in different strains. In some it is not very pronounced; thus in strain *8½* there is a decrease from 17 per cent to 9 per cent only. In others, on the contrary, it is very distinct, as in *8½ + II daughter no. 10,* where the rate decreased from 49 per cent to 14 per cent, or in *Michigan Wild + English 101,* where it dropped from 58 per cent to 25 per cent. We shall now briefly consider the various strains in which a decrease took place.

1. *Strain 121 + Cream.* This strain was composed of mice of various colors—black, sable, agouti, blackeyed and pinkeyed yellow, smoky yellow, and lavender and silver with pink eyes. Groups composed of yellow breeders had few young, while those with dark colors had a larger number. They were medium-sized and moderately tame mice. The decrease, which began in early generations and continued throughout the period of our observations, is probably due to a selection within the whole strains of certain families with a lower rate.

2. *Strain 8.* This is one of the earliest strains, and is now inbred to the 17th generation. As a result, probably, of the long inbreeding, a change has gradually taken place; while at first it was relatively prolific (although not so fertile by far as some of the more recent strains), the prolificness has decreased in the course of inbreeding and, furthermore, the individual mice now grow more slowly than in the early generations. We may in all probability attribute the fall in tumor rate to the same factors that caused the decline in prolificness and growth rate. These changes are evidently due to the effects of inbreeding.

3. *Strain 8½.* In this strain a decrease seems to have taken place from the F_4 generation on. In *European,* a fall began approximately when the fourth or fifth generation had been reached and continued in the following generations. This

TABLE 3

Decreasing tumor rate

TUMOR RATE			STRAIN	TUMOR AGE		
Previous	Intermediate	Recent		Previous	Intermediate	Recent
per cent	*per cent*	*per cent*				
42	23½	25½	*121 + Cream*	II	II–III	I
30	F_{12}–F_{64} 10		*No. 8*	F_2–F_6; ⅓ in I Period	F_7–F_{10}; one in I period; 4/10 in II period	F_{12}–F_{14}; only in III period
F_2–F_6, 34 F_7–F_{10}, 22 17	0	9	*No. 8½*	II		III–IV
16	3.6	3	*European*	I–II and I		I and III–IV
21		5.6	*European + 102 (103)*	IV		IV
65 (69)	44 (55)		*European 151 + II daughter of no. 10 (Nov. 8 strain)*	II	I + II approximately	
49 (50)		14 (20)	*8½ + II daughter of no. 10*	III		Late
17	13	0.	*(European + 103) F_1 + III daughter of no. 1)*	IV	IV	IV
55	36	23	*(European 151 + I daughter of no. 10.) (November 3) + 101*	I	I–II–III	
58	25		*Michigan Wild + English 101*	I–II (III)	I–II (III)	
50	31	33⅓	*German*	II		
41	23	20	*8 + German*	II		I–II near II age class
63	F_2–F_3 71	F_2–F_5 43	*198 (English) + (8 + German)*F_4	I	I–II	I–II

strain has always been very prolific and began breeding at an exceptionally early age, especially for several generations after the mice had been imported; and even now, in the tenth generation, its members are maturing earlier than those of some other strains. Originally they were very unhealthy, being liable to typhus and to a disease affecting very young mice, or losing their young apparently from malnutrition without any apparent disease. Owing to these conditions they finally became extinct, with the exception of the descendants of one trio, which freed themselves of typhus and in time grew to be a most vigorous strain. The *European* were very small mice when imported and for the next few generations, but finally reached medium size. They are still prolific, and not now especially subject to any disease. We see in the behavior of this strain, in so far as the incidence of spontaneous tumors is concerned, a parallel to its behavior towards propagable neoplasms; only the change was in an inverse order. At a time when their susceptibility to our transplantable mouse tumor IX increased, the rate of incidence of spontaneous growths among them decreased; and we are probably justified in attributing those changes to the same cause, viz., to an alteration in the constitution of the strain. Owing to the ravages of disease, and especially of typhus, the main strain became almost extinct and all the later European mice were the offspring of a selected family, the *Trio*. These mice were more resistant to infection, and of somewhat larger size than the other European mice; and while they were still very prolific, they apparently reached maturity not quite so early as the mixed Europeans of the earlier generations. The change in the tumor rate goes parallel, therefore, to other changes in the character of this strain, both depending in all probability on the selection of a family differing somewhat in character from the main strain.

4. Among those strains that showed a decrease in tumor rate are four hybrid strains, where *European* entered in the composition of the strain. Two of these belonged to high tumor strains, while the other two were low. Usually the decline in the tumor rate began approximately in the F_4 generation. In about the

same generation a decrease took place in the tumor rate of *Michigan Wild + 101*, in *German*, and in *8 + German*. In *198 + (8 + German)* F_4, the fall occurred even somewhat earlier. It is impossible for us at present to indicate with any degree of probability the cause of the decrease in tumor rate in these strains.

If we now inquire into the age at which tumor mice die in the various strains, we find on the whole that the earlier and later generations preserve their characteristics, although some changes do occur. In those strains where the tumor rate remained approximately constant (table 1), the following belonged formerly to the first age class: *8½ + 328*, with a substrain *782a* and the various English substrains. In all these the later generations continue in the first age class. In regard to *English Silver*, the number of tumor mice is too small to allow a definite conclusion. It is, however, of interest that a substrain of English with very low tumor rate, *Silver fawn*, belongs to the fourth age class. This confirms our former conclusions that tumors tend, on the whole, to appear at a later period in life in strains where the tumor rate is low. As for *(European + 102) + 8½* F_4, the strain belonged formerly to the IV age class, and the same holds good of the recent generations. *Cream X* now approaches the other *Cream* strains in their age class, which previously had been abnormally low. In the majority of the remaining strains, the age class has been somewhat intermediate between the first and fourth classes, and this condition is on the whole maintained, with the exception of *Cream + 10*, in which the tumors appear in later generations considerably earlier than formerly. However, in this case the number of tumor mice in our recent records is not very great. Still smaller was the number of mice observed in the substrain *240*, and a change from the second to the first age class has, therefore, very little significance in this case.

If we now consider those strains in which the tumor rate increased (table 2), we find that in the *Cream* strains the high age class remains unchanged on the whole. And even those creams in which the tumor rate rose considerably still belong to the fourth age class. However, the creams as a whole have

now to be placed in the second class. In the main strain of *German + Carter*, simultaneously with an increase in the rate the tumors now appear earlier. In one substrain, however, the tumors continue to appear late, notwithstanding the increased tumor rate.

In those strains which show a decrease in the tumor rate, we find in the majority of cases an age class essentially unchanged, viz., in *European, European + 102 (103), European 151 + II daughter of no 10, 8½ + II daughter of no. 10, (European + 103)* F_1 *+ III daughter of no. 10, Michigan wild + English 101, 8 + German,* and *198 + (8 + German)* F_4. In others the age class rose in accordance with the decrease in tumor rate. This is especially noticeable in strain *8* where about one-third of the tumors in the earlier generation appeared during the first period, while in the intermediate generations none appeared in the last period of life. A similar rise in the age class is to be noted in strains *8½* and *(European 151 + I daughter of no. 10) + 101*.

We may then conclude, on the whole, that the age class remains unchanged in the large majority of strains, and that the relation between tumor rate and age class (in the sense that a high rate is associated with the early appearance of tumors, and allow rate with their late appearance) becomes still more marked in after generations as a result of changes in the tumor age occurring in some cases. We must furthermore conclude that the age class depends upon a number of variables, especially upon the proportion of mice dying without tumors in the various age periods, and that this factor may produce certain variations in the age class in earlier and later generations of the same strains. Whenever the number of tumor mice in a certain strain is small, slight variations in the proportion of tumor mice dying in the different age periods may cause a considerable difference in the age class in which this strain is to be placed. We must not, therefore, attach too much importance to certain variations in the age class in strains where the tumor rate is low and the number of tumor mice small; it is, therefore, doubtful whether, for instance, the difference in the age class between *Silver + 10*

and *Cream + 10*, which we noticed in the earlier generations, is of more than accidental significance.

SUMMARY

1. Continued study of strains of mice in which we have established the tumor rate for earlier generations shows that in the majority of cases the rate remains the same throughout later generations. In most of these strains the constancy in the tumor rate is striking. In a few exceptional cases the rate has increased, but in a considerable number of strains there is a distinct fall.

2. If we inquire into the causes of these changes we may attribute them with great probability in certain cases to one of the following two factors: (a) to differences in resistance to disease, or in prolificness, in various families of the same strain, or to other more or less accidental factors, causing certain families with a different cancer rate to preponderate to an unequal degree in earlier and later generations; thus, an apparent change in the whole strain is due only to the effect of selection among certain constituents of the strain. (b) As the result of long continued inbreeding, certain characteristics of a strain change. The strain becomes less prolific and less vigorous, and hand in hand with this change goes a lowering of the tumor rate. This occurred in strain *8* and possibly in other strains.

3. In the majority of cases the age class in the later generations remained approximately the same as in earlier generations. In accordance with our former observations, it therefore remained characteristic of certain strains. However, while our more recent records show still more clearly the tendency of tumors to appear later in life in strains with a low tumor rate and at an early age period in those with a high rate, certain variable factors may readily produce, especially in strains with a low tumor rate, changes in the age class to which undue importance must not be attached.

REFERENCES

(1) Lathrop, A. E. C. and Loeb, Leo: Proc. Soc. Exper. Biol. and Med., 1913, xi, 34. Jour. Exper. Med., 1915, xxii, 646, and 713.

(2) Fleisher, Moyer S. and Loeb, Leo: Jour. Cancer Research, 1916, i, 331.

SOMATIC MUTATIONS AS A FACTOR IN THE PRODUCTION OF CANCER

A CRITICAL REVIEW OF v. HANSEMANN'S THEORY OF ANAPLASIA IN THE LIGHT OF MODERN KNOWLEDGE OF GENETICS

R. C. WHITMAN

From the Henry S. Denison Research Laboratories of the University of Colorado,

Received for publication, March 4, 1918

For nearly thirty years the phenomenon known by von Hansemann's term, anaplasia, has been widely used among pathologists to describe and explain the origin of cancer cells. Any well established facts, therefore, which throw added light upon the nature of the process of anaplasia itself and any possible relation it may have to cancer genesis must be important and significant.

The term anaplasia has been, however, so loosely used that it will be worth while to review at length von Hansemann's theory in order that we may start with a clear conception of what the word really means. The pleomorphism of cancer cells and the irregular, atypical mitoses so characteristic of their nuclei had been observed and carefully studied since the middle of the nineteenth century. Arnold (Virch. Arch., vol. 79, cited by von Hansemann (1)), observing irregularities in the mitotic process itself, had inferred that these might be of fundamental significance. A voluminous literature dealing with the mitoses in cancer cells and other forms of rapidly proliferating tissues had already accumulated when von Hansemann (1) undertook a further investigation in the same field. In a carcinoma of the larynx he found besides a great abundance of normal mitotic figures, large numbers of tripolar and multipolar divisions and other departures from the normal type already observed and described by earlier writers; and in addition many hyperchromatic and hypochromatic mitoses, the hypochromatism consisting not merely in a reduction of the amount of chromatin in the chromosomes but rather in an

actual reduction of the total number of the chromosomes. He reproduces a monaster with only nine chromosomes. Further search led to the finding of a hypochromatic diaster one member of which contained five chromosomes, the other eight, or possibly nine. This asymmetry of division, which he regarded as analogous to the process by which he believes that differentiation, or specialization of the cells occurs during normal embryonic development, might, he thought, take place either as the result of delay, or of complete failure of the chromosomes to split longitudinally during the mitotic process; or else through failure of the two longitudinal halves of the chromosomes to separate and pass to the opposite ends of the spindle. In one of his cases he found numerous instances in which, in the monaster stage, late in the metaphase, (or telophase), the chromosomes were found lying in pairs close to and parallel with one another, indicating that splitting had occurred shortly before. Since splitting normally occurs early in the anaphase, he inclines to the view that the asymmetry in question is due to delayed splitting, at least in some instances.

In the search for further unequal mitoses von Hansemann found, in a rodent ulcer, a diaster one member of which contained eleven chromosomes, the other sixteen. The fact that here, as also probably in the first case, the number of chromosomes in one or both members of the diaster is odd, constitutes further evidence that the asymmetry is due to the failure of splitting to occur at all in certain chromosomes, so that parts (of the chromosome) properly belonging in one-half are retained in the other.

No further mitoses were found in which inequality could be determined by actual count, but he was able with some patience to find, in every case, mitotic figures, both diaster and pluripolar forms, in which asymmetry was obviously present although the actual count could not be made. In a later paper (2), additional instances of this type are described and figured.

The occurrence of asymmetrical mitoses is then taken as established. I may add parenthetically that no one (so far as I can learn) who has concerned himself with this matter since has denied their occurrence. Ströbe (4), (cited by Hansemann

(3)), indeed, claims to have observed such mitoses in a great variety of inflammatory and regenerative conditions, in sarcomata and benign tumors, and denies that they possess any biologic significance; but v. Hansemann contends that these assertions are based on inaccurate observations.

Now, since the cells with a reduced number of chromosomes can have no other possible origin than the tumor cells, it follows that the production of such hypochromatic cells (von Hansemann, as already mentioned, applied the term hypochromatism only to cells with a reduced *number* of chromosomes—not to cells with merely a reduced *amount* of chromatin) must be accompanied by the production of cells hyperchromatic to whatever extent the former are deficient in chromosomes. The tumor thus contains cells of three sorts, viz., cells containing an excess, a normal number, and a reduced number of chromosomes, between which there is no sharp dividing line. Only the extreme cases are recognizable. The last mentioned are capable of further mitoses, but sooner or later undergo a process of necrobiosis, as is evidenced by the occurrence of certain abortive forms of mitosis (2) which we need not discuss here.

The first type, on the other hand, may be assumed to convert itself into the resting forms of the tumor cell. Such cells may continue thereafter to divide symmetrically, or may undergo further asymmetrical divisions from time to time. Hansemann (3) states explicitly that asymmetrical mitoses may possibly, even probably, occur in other than tumor tissues, and cites cases from the literature. But he maintains that no epithelial cancer occurs without asymmetrical mitosis.

In order to make clear von Hansemann's idea of the significance of this departure from the normal type of mitosis it will be necessary to follow him in a digression. The biologic properties of cells are inherent in certain definite, formed constituents of the cells, larger than a molecule, which by growth and division transmit those properties to the descendants of the cells. Hansemann believed, in 1890, with de Vries, that these constituents, then called *idioplasmae* or *pangenes*, now called *genes* or simply *factors*, are seated partly in the chromosomes, partly in the cytoplasm.

They are now known to lie exclusively in the chromosomes (vide infra). The change in views on this point does not affect the validity of what follows. During embryonic development each new stage is marked by the appearance of cells not identical with the cells from which they arise. The production of such cells is identical with the process of differentiation of the several organs, and is accompanied by a division of labor among the several kinds of cells whereby each becomes more and more dependent on the coöperation of all the rest for its own continued existence, and becomes therefore less and less capable of independent existence. This division of labor, or *altruism* as he calls it, he regards as brought about (see especially his monograph (5)), by a process of unequal, (i.e., asymmetrical) division, so that one set of functions passes into one daughter cell, and another set into the other. But the separation of functions is not absolute and complete, as otherwise even the known limited powers of metaplasia, whereby cells of one type may be converted into other but closely related types, would be incomprehensible. Those idioplasmae, or factors, which dominate the character of the fully differentiated cell, he calls (5) *chief plasmae* (*Hauptplasmen*) and those factors which become latent, so as to exercise no visible effect on the functional activity of the cell under normal circumstances, he calls *secondary plasmae* (*Nebenplasmen*). The whole process by which the embryonic cell develops into the fully differentiated cell, he calls *prosoplasia*. The reverse process, by which a cell loses more or less completely its specialized functions, and its altruistic relation to the rest of the organism, i.e., gains in its capacity to live independently, he calls *anaplasia*.

It is at this point that von Hansemann's argument becomes a bit involved. Anaplasia is defined as a process of undifferentiation. Ribbert (6, p. 451 ff.), who comments on the frequent misunderstanding of von Hansemann's theory, himself falls into obvious error. von Hansemann thinks, he says, that "through the loss in differentiation, changes arise in the cells which he includes under the designation 'anaplasia.'" That is incorrect. Anaplasia means, not changes due to undifferentiation but the undifferentiation itself. Furthermore, von Hansemann is ex-

plicit (5) in his statement that the anaplastic cell is not an embryonic cell in the sense that it is identical with any cell at any time present in the normal embryo. Anaplasia is not a reversal of the normal direction of ontogenesis. The embryonic cell is a prosoplastic cell, and anaplasia begins where the embryonic cell leaves off. Anaplasia produces a cell different from any cell at any time normally present in the body.

The difficulty in understanding just what is meant by anaplasia arises primarily from hasty reading, but in large part also, it seems to me, from an unfortunate selection of descriptive terms on v. Hansemann's part. For, after all, one can hardly be blamed for visualizing *undifferentiation* as precisely that reversal of the normal course and direction of ontogenesis which anaplasia is not. If he had spoken of anaplasia as a process of *re-differentiation* much of the misunderstanding would have been avoided.

The asymmetrical mitoses then, according to von Hansemann, since the smaller of the unequal pair of daughter cells sooner or later dies, while the larger becomes the tumor cell, result in the production of cells whose chromosomes vary in number from cell to cell. Some of the tumor cells contain, indeed, an increased number of chromosomes and von Hansemann thinks (2) that it is the function of the pluripolar mitoses so often found to restore the normal number. Every loss of a chromosome, or group of chromosomes, involves the loss, on the part of that cell and its descendants, of those powers and functions the plasmae or genes for which were carried by the lost chromosomes; and, per contra, the retention in one cell of chromosomes which normally should have passed to another cell, involves the heaping up, the doubling, of all genes carried by the chromosomes thus retained. This cell, the cancer cell, is thus a "new kind of cell." In modern terminology it is, strictly and literally, a mutated cell. Since the process is, or at least may be, repeating itself from time to time, and here and there, in a tumor, it follows that the tumor cells themselves are by no means all alike in their biologic properties; that, on the contrary, an ever recurring process of mutation is taking place, with a tendency, however, to deviate more and more from the normal type. This explains why metastatic tumors,

for example, are often more, but never less, malignant than the primary tumor, as well as other related phenomena of tumor growth.

The anaplastic cell then is one in which, through some unknown agency, a progressive disorganization of the mitotic process occurs, which in turn results in the production of cells that are *undifferentiated* in the sense that those functions last to be acquired, most highly specialized, and perhaps most dependent for their continued functional efficiency on a continued altruistic relation to the other body cells, are *more* or *less* lost; but *redifferentiated* in the sense that the cancer cell is not at all an embryonic cell, but is a new biologic entity, differing from any cell present at any time in normal ontogenesis. But it must be remembered always that this new entity displays no characters absolutely and completely lacking in the mother cell—no characters created de novo, and out of nothing. Its changed behavior depends on exaltation of some qualities, and depression of others, all at least potentially present in the mother cell.

I would pause here merely to observe that numerous other theories of cancer genesis, Hauser's (7) "new cell-race," Butlin's (8) conception of the cancer cell as being itself the parasite of cancer, Oertel's (9) theory of the double nature of the nucleus, etc., to which references will be found in the bibliography, are merely variants of von Hansemann's parent idea, and differ from the last chiefly in terminology.

von Hansemann makes no attempt to account for the invasive tendency of malignant tumors beyond calling attention to the motility of cancer cells, first observed, he says, by Grawitz, and subsequently confirmed by many observers, including himself. It is also conceivable that the production of metastases and invasive growth depends merely on the passive transport of cancer cells in the body fluids. Nor does he make any attempt to explain the ultimate etiology of cancer.

Ribbert (6) opposes von Hansemann's theory on the following grounds:

1. As already mentioned Ströbe, on insufficient evidence according to von Hansemann, asserts that he has observed asym-

metrical mitoses in a great variety of non-cancerous lesions. Granting, for the sake of argument, the accuracy of Ströbe's observations, it does not follow that asymmetry must have the same significance in all cases. The complexity and delicacy of the structures concerned is so great that, as we now know (vide infra), the most varied results might easily follow an interference with the mitotic process, depending on differences in the character and seat of that interference which we are utterly unable to detect by direct observation.

2. On the point that anaplasia means not merely the return to an earlier stage of development but a loss of differentiation such that the cell becomes something which normally is found in the body at no stage of development, Ribbert contends that the very polymorphism and variation in size and structure of the nucleus, indicate that the cancer cell is not a cell which has attained to a characteristic condition through anaplasia. The cancer cell must, he urges, be a definite fixed entity (*etwas Eigenartiges sein*), i.e., it can not be a variable, a lawless thing. On the contrary its polymorphism arises simply from its abnormal environment.

To this it may be answered that there is, of course, absolutely no proof that all the cells of a given cancer have a like constitution; and since we would all agree with Cicero, that whatever is has a cause, we would concede that *something* in the environment is responsible for the change in the cell; but this something, insofar as it applies merely to the effect of environment (pressure, food, oxygen, etc.) on the behavior of the cancer cell in the fully developed tumor, is irrelevant except that such conditions might act to induce or accelerate further anaplastic changes. So far as it concerns the effect of the environment in setting in motion the initial anaplastic change, it is a question of etiology again quite irrelevant to von Hansemann's argument.

3. In any except the most atypical and malignant cancers the degree of departure from the normal type is not great enough to furnish a basis for the concept of anaplasia. von Hansemann (10) has himself pointed out that the loss of function in cancer cells is not often as complete as the generally accepted notion

would imply, and insists that the whole process of anaplasia is a gradual and progressive one, and that the loss of function increases pari passu with the anaplasia. Ribbert appears to have reached the point of view just indicated by visualizing von Hansemann's concept of the anaplastic cell as a cell exhibiting an extreme departure from the normal type, and excluding cells showing minor degrees of departure—an attitude quite out of harmony with the whole course of v. Hansemann's reasoning. Ribbert apparently thinks of anaplasia as a return to an earlier stage of embryonic development. von Hansemann thinks of it as a process carrying the cell in some entirely new direction—a direction, moreover, which is not the same in all tumors, nor even constant in the same tumor. Ribbert's total failure to grasp this point is clearly seen in the following paragraph (p. 455):

> Whatever striking peculiarities (which were absent in the earlier beginning stages) the cancer cell in the fully developed tumor may show, are the result of changes occurring in the fully developed carcinoma; and whatever deviations from the normal type are present at the very beginning of cancer development, represent merely a loss of differentiation, such as we see also under other conditons. *There is no justification in either case for inferring that anaplasia has occurred.*

v. Hansemann constantly uses the term anaplasia interchangeably with undifferentiation, except that, as above noted, anaplasia implies undifferentiation in some new direction or directions.

4. Anaplasia offers no explanation of the invasive growth. A mere tendency to increased growth, due to anaplasia, would cause merely a local hyperplasia, in which the cells would grow in the direction of least resistance, or toward the free surface. Ribbert assumes that the inflammatory changes always present in the underlying connective tissue loosen the latter and thus favor, mechanically, infiltration by the tumor cells. But this raises a double difficulty. The assumption that inflammatory changes favor rather than hinder the cancerous invasion sharply challenges our concept of the biologic significance of the inflammatory reaction. Ribbert meets this by arguing that since the reaction

is excited by cell products not foreign to the body it remains sluggish and lags behind, in intensity, its exciting cause.

What we chiefly see in a primary carcinoma is a cellular infiltration and moderate proliferation of the fixed elements. These processes are not adequate to check an epithelial infiltration; on the contrary they loosen the connective tissue, and thus create a lowered resistance, a sort of cavity formation, which sets in motion the downgrowth of the tumor cells (p. 493).

But it is at least difficult to understand how any conceivable change in the connective tissue can ever lower the resistance offered by it to a point less than that on the free surface. We have to assume that, if the connective tissue is the prime cause of the invasive growth, it is so by virtue of an actual positive attraction, not merely a negative lowering of the mechanical resistance: which brings us back to the starting point where our ideas of the fundamental significance of the inflammatory process are violated.

5. And finally, in his discussion of Hauser's new cell race theory (p. 458), Ribbert raises a point which applies with equal force to von Hansemann. The cancer cell cannot be a "new race of cells," because, "How can a cell arise which lies *entirely* outside the phylogenetic series? How can cells acquire *entirely* new properties?" (Italics mine.) I have urged already that the cancer cell exhibits no *entirely* new properties. Further he says "All development is continuous. If, in the course of phylogenesis, new sorts of cells are produced, they are produced always gradually, and are never elements injurious to the organism"—a statement which Ribbert would hardly make save in the heat of an ex parte argument. And again (p. 477), he maintains that new biologic properties arising in the course of ontogenesis can only arise in accordance with fixed laws of inheritance; that if we assume that properties arise independently of such laws, they can do so only as the result of adaptation to changes in the environment. Applied to tumors, this means that tumor cells can arise only under those influences which determine tumor formation. Under no conceivable circumstances could cells learn to

live under abnormal conditions until after those conditions have acted upon them. Stimuli call forth in cells and tissues, it is true, insofar as these are not injured, adaptive changes, but the adaptation is to the stimulus, not to something else, hence not to the cause of the invasive growth. Stimuli therefore cannot produce destructive properties in the cells. The ability to live in other tissues, and to invade them, could only be acquired under the conditions existing during the growth itself. Hence a primary change in the cell leading to invasive growth cannot exist.

Now it is true that mutations can arise only as the result of some change or other in the environment. To assert otherwise would be to assert that things happen without a cause. But it is not true that the net result of the adaptive change is never injurious to the organism. Are hemophilia, and Daltonism, and intrinsic epilepsy advantages or disadvantages? Yet these heredity conditions must in every case have arisen in the first instance as a mutation in response to some change in the environment. Nor is it true that the adaptive reaction always stands in direct and obvious relation to the environmental change which produced it. The reaction may be excessive or involve remote effects quite different from the primary purpose to be served. If I may be permitted to cite examples outside the domain of heredity (if they are outside), we should, I dare say, all agree that the inflammatory reaction is on the whole designed by nature to serve a beneficent end, but the violence of the reaction in a pneumonia may render the reaction itself more dangerous to life than is the cause of the reaction; and again, the healing of multitudinous minute injuries in the kidney leads finally to the death of the patient. The error has a double source: Ribbert is obsessed with the idea that adaptation must always be protective and must always show a direct and obvious relation to its cause; just how erroneous this is will become apparent from what follows. And secondly, he entirely fails to keep separate in his own mind two distinct issues. He himself is chiefly concerned with the factors that lie back of and induce the cancerous change in the epithelial cell; von Hansemann, on the other hand, is interested in the intracellular mechanism by which that change comes into being.

Before I proceed I should like to pause long enough to point out that, if the distinction just drawn be kept clearly in mind, no irreconcilable difference is found to exist between Ribbert and von Hansemann. Each has for thirty years vigorously and sometimes heatedly championed his own view as opposed to that of the other. Yet, except perhaps in certain details, neither view excludes the other.

Morgan (11) and his associates have observed more than 125 mutations occurring "spontaneously" under the uniform conditions of laboratory cultivation, in the fruit-fly, *Drosophila ampelophila*. This fly has three pairs of autosomes and one pair of sex chromosomes. The last differ morphologically from the

FIG. 1. CHROMOSOMES OF THE D. AMPELOPHILA

Female at the left, male at the right. The X chromosomes at the bottom appear as straight rods. The XY complex in the male at the right is recognizable by its different morphology. Figures 1 and 2 are reproduced by permission of the author from Morgan's "A Critique of the Theory of Evolution," Princeton University Press, 1916.

autosomes, and, in the male, from each other, the female having typically two "X" chromosomes, and the male one "X" and one "Y" chromosome. The "Y" chromosome is not the carrier of any known hereditary factors, and indeed has no known function beyond the fact that males lacking it are sterile (fig. 1), (Bridges, 12). By methods the description of which may be omitted here, it was shown that the factors of inheritance, or genes, have their seat in the chromosomes; that the several genes are arranged in a row, or rows, in the chromosome, like beads on a string; and that each gene has a definite fixed locus in the chromosome, which can be determined and mapped (fig. 2). I select for comment a few mutations which illustrate points with which we are concerned. Among these is the character *eyeless* (fig. 2)

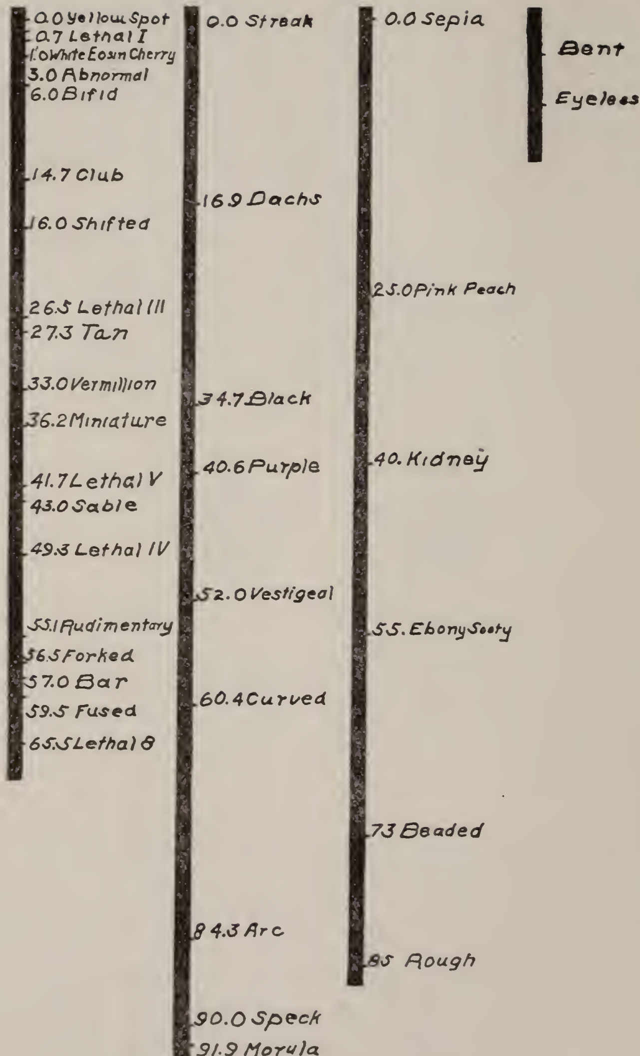


Fig. 2. Chart of the Chromosomes of D. ampelophila, Showing the Best Known of the Mutations Observed

The figures are the unit distances apart, and the words are the laboratory designations of the several mutations. The chromosome at the left is the X chromosome and the short chromosome at the right is the fourth chromosome indicated by the pair of dots in figure 1 (Morgan).

in the fourth chromosome. This change took place at a single step, but is variable, some of the individuals showing more or less rudimentary organs. Morgan comments (p. 67):

> Formerly we were taught that eyeless animals arose in caves. This case shows that they may also arise suddenly in glass milk bottles, by a change in a single factor.

There also appeared in the cultures some twelve modifications of the shape and size of the wings, which might easily be arranged in a series culminating in a wingless insect. But as a matter of fact each of these arose independently of the others and was shown to be due to distinct factors. Five of these have their loci in the first chromosome, and seven in the second chromosome. Moreover factors not infrequently produce more than one effect. For example,

> A mutant stock called "rudimentary wings" has as its principal characteristic very short wings. But the factor for rudimentary wings also produces other effects as well. The females are almost completely sterile, while the males are fertile. The viability of the stock is poor . . . . The hind legs are also shortened.

Again the factor producing one of the wing changes above mentioned is a dominant so far as the shape of the wings is concerned and is at the same time a recessive lethal factor. As this character is sex-linked, no males carrying this factor (they being haploid as regards their sex chromosome) survive. In another case a factor causing a difference in the body color, called tan, also produces a loss of positive heliotropism.

A gene which causes duplication of the legs was found and it was further shown that this effect takes place only in the cold. Flies grown at ordinary temperature have normal legs. In another culture a number of flies were found whose wing pads failed to expand, and two bristles were lacking on the side of the thorax. It was found that the wing anomaly was present in only 20 per cent of the flies of this strain, apparently because it was an "environmental effect peculiar to the stock," whereas the absence of the bristles was constant. Thirty different

factors were identified which have an influence on the eye color. "It is probable that there are at least as many normal factors that are involved in the production of the red eye of the wild fly."

A considerable number of lethal factors (eight including those listed by Bridges (12)) has been located in the X chromosome. One of these, lethal 7 (Bridges, loc. cit.), .

causes the death of the individual at the mature larval stage. Those larvae which are about to die can be separated. . . . . because . . . . . . when half mature [they] leave the food and wander about on the surface of the culture bottle. Furthermore, while still young, one or more intense black specks appear in their body cavities. As the larvae become older this character . . . . . becomes more conspicuous, so that one can easily pick out those cultures in which the mother was heterozygous for lethal 7 . . . . . Lethal 7 is then a larval character of such virulence as to cause a change in the instincts of the individual and finally cause its death.[1]

[1] It is not without interest in this connection to note that at least one lethal factor is known in man. Daltonism and hemophilia are sex-linked characters, and Daltonism, according to Rössle (13) when total is, in the male, recessive; when partial, dominant; in the female, it is recessive. This is not the case, as Morgan shows, for according to von Winniwarter (14) the constitution of the sex chromosomes is XX in the female, X in the male; according to Wieman (15) the constitution of the human sex chromosomes is XX in the female, XY in the male, exactly like the Drosophila. The mechanism of transmission is the same in either case, as follows:

Mother	*Father*
X′X	X (or XY)
At the reduction division two sorts of ova are produced, viz.,	At the reduction division two sorts of spermatozoa are produced, viz.,

$$\begin{array}{ccc} & X \longleftrightarrow X & \\ \text{and} & \times & \\ & X' \longleftrightarrow O \text{ (or Y)} & \end{array}$$

Offspring

(a)	(b)	(c)	(d)
XX	XX′	XO (or Y)	X′O (or Y)

The X′ is the sex chromosome carrying the recessive factor for Daltonism. The arrows indicate the possible combinations of ovum and spermatozoon in fertilization.

It is apparent that half the daughters will be normal (a); half will be apparently normal but like the mother, capable of transmitting the condition (b), since the

Bridges (12) also describes, and Morgan (11) offers positive cytological proof of, the occurrence of non-disjunction, i.e., the failure of homologous chromosomes to separate, in mitosis, to the opposite poles of the spindle, with the resulting production of unexpected Mendelian results. This, it will be recalled, is precisely one of the mechanisms by which von Hansemann conceived that anaplasia might occur.

Among the factors most significant and important to the present discussion are the *modifiers*. Morgan cites a number of cases to show that so-called *unit characters* are not infrequently the net result of the interaction of several genes, and that in

effect of the recessive factor is offset, so far as that individual is concerned, by the corresponding dominant allelomorph in X; half the sons will be normal (c) and half will be color blind (d), since, whether a Y chromosome is present or not, no dominant allelomorph is present to offset the recessive for Daltonism in X^1 (v supra, p. 139).

A color blind female can only be produced by crossing a female transmitter of Daltonism like the mother above with a color blind male, as follows:

Mother	*Father*
XX′	X′O

At reduction two sorts of ova— At reduction two sorts of spermatozoa =

$$\begin{array}{ccc} & X \longleftrightarrow X' \\ \text{and} & \times \\ & X' \longleftrightarrow O \end{array}$$

Yielding offspring as follows:

(a)	(b)	(c)	(d)
XX′	X′X′	XO	X′O

a = apparently normal transmitter like the mother
b = color blind female
c = normal male
d = color blind male

The male is haploid as to the sex chromosomes; the female is diploid, hence sex-linked recessive characters behave like recessives in the female, but like dominants in the male.

Hemophilia is exactly on all fours with the above, except that, according to Rössle (loc. cit. p. 36) "The occurrence of genuine hereditary hemophilia has never yet been demonstrated in women." Since color blindness does occur in women, the failure of hemophilia to occur can only be explained on the assumption of a miraculously happy chance, or on the assumption that the hemophilic gene, when doubled, acts as a lethal.

some cases a factor is entirely without effect except in the presence of other factors (p. 163, et seq.).

And finally Bridges (16) has shown that a definite region of the X chromosome, including the loci of several factors, may entirely disappear, may become, in other words, "genetically non-existent," and that this loss when present in the (haploid) male or in both X chromosomes of the (diploid) female may render the individual non-viable. He says,

> In general it is to be anticipated that cases of deficiency should act as lethals, since it seems probable that any very extensive piece (of a chromosome) includes a locus vital to the animal.

Furthermore, it has been shown by Bridges (12) that an entire chromosome or a part of a chromosome may be lost when a chromosome "lags" on the spindle during mitosis, though why a chromosome should lag behind its fellows is of course not known. It can only be surmised that, through some disturbance, the character of the chromosome substance undergoes a change, analogous, perhaps, to the change undergone by endothelial cells in inflammation, whereby the cell, although it shows no visible alteration, opposes a greatly increased frictional resistance to the flow of the blood. If such a change does take place we may imagine the affected chromosome as adhering to the spindle and thus lagging behind because it is, so to speak, mired. A similar process was described by von Hansemann (loc. cit. 5) as occurring in cancer cells, and was thought by him to constitute an additional mechanism by which anaplasia might occur.

It has then been proved, on the one hand (Morgan, Bridges), that changes in the chromosome of an exceedingly delicate character may produce profound and far reaching changes in the structure and character of the individual; and that such changes arise without apparent cause, without waiting upon any visible changes in the environment. Roughly the law of heredity, so far as the initiation of mutative changes is concerned, lies, so far as can be observed, in their very lawlessness, though of course actually that is not the case. On the other hand, von Hansemann has furnished conclusive evidence that the cancer cell undergoes

changes in its chromosomes of far greater extent and severity than the slight changes shown to produce mutations. It may, I think, be regarded as proved that the changes in the cancer cell described by von Hansemann are precisely of the character demanded by the available data to explain the change in behavior of the cancer cell as compared with the normal parent cell. The trouble is, indeed, not that the changes observed in cancer cells prove too little, as Ribbert argues, but that they seem rather to prove too much. For example, Bridges' remark, already quoted, that "in general it is to be anticipated that cases of deficiency should act as lethals, since it seems probable that any very extensive piece includes a locus vital to the animal," raises a difficulty here, since the cancer cell is obviously enormously deficient in Bridges' sense. But Bridges is speaking of inheritable changes, i.e., of modifications presumably involving equally every cell of the body. Such a change, when it involves a "locus vital to the animal" would obviously have quite a different significance when it involves a limited group of cells. Nor need such a change be lethal to that group of cells, especially when those cells, by the very nature of the mutation, have acquired the greatly increased power to stand alone.

But clearly anaplasia, as I said, proves too much. I incline strongly to the view that we are on safer ground in insisting, not on the loss of entire chromosomes and groups of chromosomes which anaplasia is said to produce, but rather upon the profound general disturbance of the mitotic process which is obviously present, and the abundant opportunity for modification of factors, and the production of new factors, which is thus afforded. For if von Hansemann's explanation of asymmetrical mitosis as due to delayed or absent longitudinal splitting be correct, it is apparent that the smaller of the asymmetrical pair of cells would contain only those chromosomes which have split in due season; and the larger of the pair would contain the homologue of those chromosomes, plus all those chromosomes which have failed to split. Since the larger cell is assumed to be the tumor cell, the net result of the process would be, not a loss of hereditary factors

to the tumor cell, but a heaping up, a doubling, of all those that remain behind during mitosis, in the tumor cell.

I quote here a remark of Morgan's (loc. cit., p. 162),

> By crossing different wild species, or by crossing wild with races already domesticated, new combinations have been made. Parts of one individual have been combined with parts of others, creating new combinations. *It is possible even that characters that are entirely new may be produced by the interaction of factors brought into recombination.* [Italics mine.]

This brings us to the chief difficulty, viz., the question whether a somatic mutation can occur, i.e., a mutation involving only somatic cells, and not the germ cells, therefore not inheritable. Most of the observations reported on this point have to do with plants—which makes it important to remember that the mechanism of Mendelian inheritance is identical throughout the plant and animal kingdoms. Cockerell (17) has described a deficiency in the rays of a sunflower, involving equally and in the same relative position, all the flowers produced by the plant, which he interprets as a somatic mutation. But no experiments were made to determine whether the mutation was purely local, or involved the constitution of the whole plant. A somewhat different condition is presented by the "bud variations" occurring in plants, and consisting in the production of unlike flowers on different branches of the same plant, e.g., a yellow chrysanthemum carrying white flowers on one branch. A recent discussion of this phenomenon is that of East (18). It is obvious that, since the color of the flower is known to depend on definite factors, the development of a branch carrying flowers of a color different from that of the flowers on the other branches could only arise through the failure of the factor to pass over, during mitosis, into the cell from which that branch developed. The branch, in other words, represents a somatic mutation due to asymmetrical mitosis. Nor are similar instances lacking in the higher animals. Every one has seen dogs and horses whose eyes differ in color. The color of the eye is known to depend on Mendelian factors. There is therefore no other conceivable explanation than that the

anlage of the lighter eye has "dropped" the color factor, or the darker eye has "picked up" a color factor present or absent, respectively, in the other eye. Clearly also this is a somatic mutation, based on asymmetrical mitosis. Certain anomalies of skin pigmentation may also be cited here, though, it must be admitted, on less secure grounds. But, since the production of melanin in the skin, as in the eye, depends on Mendelian factors, the disappearance of normal pigment in vitiligo, in certain scars, etc., can be most plausibly explained as due to the dropping of that factor; and, on the other hand, an excess of pigment, in naevi and some scars, may be due to the picking up of pigment genes. Where the pigment is more or less symmetrically disposed, as in freckles, which are listed by Rössle (loc cit.) among the hereditary anomalies of the skin, there is of course less ground for assuming a purely somatic variation, and in Addison's disease the pigmentation seems to depend on a disturbance of metabolism which may or may not involve a change in the Mendelian factors for color.

I would not be understood as maintaining that the status of all the preceding pigment anomalies as somatic mutations is proved, though I believe that the status of those referred to in the eye is fairly clear. Granting that conclusive evidence of the occurrence of somatic mutations in man is lacking, I submit that no inherent impossibility, or even improbability, of such an event exists. Mutations must arise in the first instance by a change in the factors either of one or the other of the sexual cells which unite to produce the individual, or of the cell which is the product of that union. If such a change can take place in these cells there is no apparent reason why it cannot take place in a cell anywhere in the body. Those who believe that cancer is inheritable will, of course, experience no difficulty on the score of somatic mutation.

But cancer may be inheritable in quite another sense. It is a matter of common knowledge that highly hybridized, or mongrelized, plants and animals are prone not to breed true. The bud-variations in plants already referred to are much commoner in hybrids (East, loc. cit.). Extreme examples are furnished by the potato and dahlia, neither of which can be bred true from seed.

In certain of the dahlias (I may mention the varieties known as *Mrs. H. J. Jones* and *Fritziman*), this instability is so great that flowers developing on the same plant often differ from one another to a greater or less extent. Parallel with this fact is the general impression that cancer is less frequent among aboriginal races than among the civilized and highly hybridized races. I have compiled the following table from Hoffman (19) showing the cancer rates for various races. In general the rates increase with the generally accepted degree of mongrelization. My first attempt to secure adequate confirmation of this impression quickly convinced me that the confirmation would involve such an extended discussion as to carry me beyond the possible limits of a single paper. I have therefore reserved this question for separate treatment. I would, however, call special attention to the marked difference in the cancer rate of the Chinese, Japanese, and ourselves; in that of the negroes in the United States and the native blacks of Johannesburg; the wide variations in the rates for the several races of Hungary listed in the table; and the tremendous difference indicated between the rate given for Iceland on the one hand and Finland and Switzerland on the other.

	Rate per 100,000
Chinese in Hongkong, 1901–1912	6.07
Europeans in Hongkong, 1901–1912	50.107
Japan as a whole, 1899–1911	44.0 to 66.9
United States as a whole, 1900–1916	63.0 to 81.8
Negroes in the United States, 1910–12	56.1
Johannesburg, South Africa, 1909–1911:	
Whites	51.9
Asiatics	46.5
Native blacks	14.5
North American Indian	"extremely rare"
Constantinople, 1908–1912	
Mohammedans	22.6
Jews	44.2
Armenians	56.3
Greeks	56.1
Others	46.7
Austria, 1907–1911:	
Serbo-Croats	31.3
Polish Ruthenians	33.8
Slovene	47.9
Bohemian	102.0
German	110.0

Hungary, 1901–1904:	
Ruthenian	4.6
Rumanian	11.3
Croats	17.9
Serbian	19.3
Slovak	28.5
Magyar	44.1
German	56.4
Others	32.5
Iceland, 1908:	
Males	15.0
Females	39.7
Finland, 1909	100.0
Switzerland, 1881–1912	109.7 to 125.9
Italy, 1900–1912	52.2 to 64.7
Spain, 1900–1912	39.3 to 55.5
Portugal, 1902–1910	stationary at 22.7

It is possible, of course, that a part at least of the apparent variation is due to variation in the proportion of cases which escape diagnosis. But this possibility would seem to be unimportant in such regions as Hungary, and perhaps Constantinople. Caution is also needed to avoid drawing inferences from a mere post hoc propter hoc argument. But in view of the considerations already urged, the table is at least suggestive. The situation throws an interesting light and affords biologic justification for the instinct of race purity, if we assume that the intermarriage of races, by introducing a great mass of new factors of heredity, introduces also an element of instability by which the tendency to cancer is increased.

Finally I wish to acknowledge my debt, and express my gratitude, to my colleague, Professor Cockerell, of our department of biology. His suggestion started me in the course of reasoning which led to the foregoing essay, and he has constantly aided me with suggestion and advice, in dealing with the biological questions involved. I had hoped that he would join with me in authorship, but he has, modestly, or prudently, declined that responsibility.

REFERENCES

(1) von Hansemann: Virchows Arch., 1890, cxix, 299.
(2) von Hansemann: Virchows Arch., 1891, cxxiii, 356.
(3) von Hansemann: Virchows Arch., 1892, cxxix, 436.
(4) Ströbe: Cited by von Hansemann. Zieglers Beitr., 1892, xi, 10.
(5) von Hansemann: Studien über die Spezificität, den Altruismus, und die Anaplasie der Zellen, Berlin, 1893.
(6) Ribbert: Das Karzinom des Menschen, Bonn, 1911.
(7) Hauser: Das Zylinderzellen Karzinom, Jena, 1890; Virchows Arch., 1895, cxli, 485.
(8) Butlin: Lancet, London, 1911, clxxxi, 1535.
(9) Oertel: New York Med. Jour., 1907, lxxxvi, 14.
(10) von Hansemann: Ztschr. f. Krebsforsch., 1906, iv, 565.
(11) Morgan: Critique of the Theory of Evolution, Princeton University Press, 1916.
(12) Bridges: Genetics, 1916, i, 1 and 107.
(13) Rössle: Aschoff's Path. Anat., 1911, i, 35.
(14) von Winniwarter: Arch. d. Biol., 1912, xxvi, 91 and 189.
(15) Wieman: Am. Jour. Anat., 1917, xxi, 1.
(16) Bridges: Genetics, 1917, ii, 445.
(17) Cockerell: Jour. Heredity, 1917, viii, 467.
(18) East: The Amer. Naturalist, 1917, li, 129.
(19) Hoffman: Mortality from Cancer throughout the World, Newark, 1915.

THE COST OF CANCER IN NORWAY[1]

F. G. GADE

Received for publication, May 18, 1918

The various disasters to which death and disease give rise have an economic side, which occasionally makes itself strongly apparent. The author has therefore tried to calculate the economic losses to Norway brought about by deaths from the cancerous diseases (carcinoma and sarcoma) in that country.

The calculations are based on one side upon the mortality statistics of Norway for the years 1902–1911, reporting 22,093 deaths caused by malignant tumors; and on the other, upon the valuation of Norwegian lives in 1912, as published by Mr. A. N. Kiaer in Statsovekonomisk Tidsskrift, 1913.

Mr. Kiaer, then director of the Norwegian Statistical State Bureau, took for his starting point the average wage and income-tax within all classes and ages of the population, and calculated from these data the capital values of individual lives at the various ages, by discounting the probable income during the remainder of life.

As these valuations were calculated for 1912, however, the results would give too high values when applied to the period 1902–1911, for wages, and consequently the value of life in Norway during this period, have risen by at least 1 per cent a year; it has been necessary, accordingly, to reduce Kiaer's results to the standard of 1906, representing the medium year of the decade 1902–1911.

Concerning the age and sex distribution of the 22,093 deaths from malignant tumors, columns 1 and 4 of the subjoined table will give necessary information. It may be mentioned that

[1] This paper is an abstract, by the author, of one published by him in Norsk Mag. f. Lægev., March, 1918, page 325.

the number of deaths at different ages during single years shows only slight variations throughout the period under discussion.

Loss of capital through cancer mortality 1902–1911

Age	MALES			FEMALES		
	(1) Number of deaths 1902-11	(2) Individual capital value (1906)	(3) Loss of capital	(4) Number of deaths 1902-11	(5) Individual capital value (1906)	(6) Loss of capital
		kroner	*kroner*		*kroner*	*kroner*
0–5	52	4,175	217,100	25	2,437	60,925
5–10	20	5,882	117,680	16	3,424	54,784
10–15	13	7,882	102,466	19	4,545	86,355
15–20	36	9,774	251,864	32	5,375	172,000
20–25	29	11,126	322,654	32	5,788	185,216
25–30	57	11,941	680,637	81	6,000	486,000
30–35	78	11,908	928,824	138	5,971	823,998
35–40	173	11,435	1,978,155	292	5,658	1,652,136
40–45	300	10,628	3,188,400	546	5,104	2,786,784
45–50	549	9,404	5,162,796	797	4,326	3,447,822
50–55	965	7,856	7,581,047	1,111	3,502	3,890,722
55–60	1,277	6,094	7,782,038	1,326	2,674	3,545,724
60–65	1,619	4,308	6,974,652	1,512	1,858	2,809,292
65–70	1,718	2,769	4,755,142	1,551	1,205	1,868,955
70–75	1,674	1,507	2,522,718	1,503	699	1,050,587
75–80	1,362	670	912,540	1,246	355	442,390
80–85	609	288	175,392	669	164	109,716
85–90	234	127	29,718	332	82	27,224
90–100	41	34	1,394	59	32	1,888
	10,806		43,685,217	11,287		23,502,522

Total kr. 67,187,739 = $18,000,000.

In a former paper, a summary of which has been published in this Journal (1918, iii, 107) the author has shown that the cancerous diseases in Norway attack males and females in exactly the same proportion; also, that in both sexes the highest number of deaths from malignant tumors occurs between sixty to seventy-five years, during which period there are recorded nearly half of the total number of all deaths from cancer. This fact will be evident from the table above, which also shows that women from thirty-five to fifty are considerably more liable to cancer than men (cancer of the breast and the uterus in particular having

its maximum during this period); in men, the mortality is higher at sixty to eighty-five years (cancer of the stomach and of the skin and lips). For the ages above eighty-five years, the greater number of females surviving at this age explains the greater number of deaths from cancer in women.

As seen in columns 2 and 5 of the table, the capital value of an individual reaches its height in the years between twenty and forty, and particularly between twenty-five and thirty-five years. For males, this value has been calculated by Kiaer to be about kr. 12000 (= $3,217, a dollar being equal to kroner 3.73); for females, about kr. 6000 (= $1,609), the capital value of men amounting through all age periods to about twice as much as that of women, the worth of the household work performed by the latter having been taken into account by estimation. That this economic valuation of the various ages and the two sexes can by no means give a true and valid standard for the real worth of the individuals and the sexes, need not be specially pointed out; the individuals are here considered exclusively as income-producing members of society.

From these calculations we find, for the period in question, that the total loss by deaths from cancer is among

Men	kr. 43,700,000	=	$11,700,000
Women	kr. 23,500,000	=	6,300,000
Total	kr. 67,200,000	=	$18,000,000

Consequently it averages *for each single year kr. 6,720,000 = $1,800,000,* for a population of scarcely 2,500,000 inhabitants.

This total, however, must be understood to cover only the direct loss of life-capital, and must be considerably increased if other sources of economic loss through the diseases in question are to be considered. Moreover, the constantly increasing number of deaths from cancer reported in Norway involves a correspondingly increasing loss. Again, it must be taken into consideration that among the 12.5 per cent of all deaths in Norway, the causes of which are not diagnosed, there will probably be found a proportionate number of deaths from cancer.

Finally, the rising wages and capital value of life in Norway will constantly augment the economic loss brought about by comparatively early death.

All things considered, *the total yearly economic loss through cancerous diseases in Norway may at present be estimated at about 10,000,000 kroner, or $2,680,000.*

PRIMARY SPONTANEOUS TUMORS OF THE TESTICLE AND SEMINAL VESICLE IN MICE AND OTHER ANIMALS

XII, STUDIES IN THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

MAUD SLYE, HARRIET F. HOLMES, AND H. GIDEON WELLS

From the Otho S. A. Sprague Memorial Institute and the Department of Pathology of the University of Chicago

Received for publication, April 8, 1918

Tumors of the testicle would seem to be uncommon in mice, for we have been unable to find even a single case reported in the literature, although they have been described in other species of lower animals. Thus, among 103 primary tumors observed by McCoy (1) among 100,000 rats killed in plague work, there was one described as an "angiosarcoma" of the testicle, without further details. Other series of tumors in wild rats (Woolley and Wherry (2), Beatti (3)) do not record cases of testicular tumors. Caspar (4) says:

> Carcinomas of the testicle, often described in horses and dogs, form sometimes soft, sometimes hard tumors, in which not rarely single portions are formed differently. Through mucoid and colloid degeneration of the cell nests, cysts with gelatinous contents may be formed.

Infiltration along the spermatic cord and lymph-node metastases are often observed. Horses appear especially to develop testicular tumors, particularly if we consider the relatively small number of old animals that have not been castrated. In Japan, where this operation is less often performed than in Europe, equine testicular tumors are most abundant. Thus Kimura records the finding of 49 such growths among 142 tumors observed in 77,224 slaughtered horses. This may be compared

with the figures given in the census statistics on the mortality from cancer in the registration area of the United States, which shows that of 52,420 cases of malignant tumor there were but 121 recorded as arising in the testicles, or 2.3 per thousand; as 21,282 of the cancer cases were males, the proportion of testicle tumors is 5.8 per thousand of all tumors in males. Kimura studied in detail 12 specimens of equine "orchidoblastomas" varying in size up to 7500 grams weight; all were unilateral, and in at least five cases there were metastases in the spermatic cord and the inguinal and lumbar lymph-nodes. He describes their anatomic characteristics as follows:

1. Tumors usually develop without altering the normal shape of the testis. The albuginea is somewhat thickened, showing more or less engorgement of blood-vessels on its surface and expanding with the growth of the tumor, and may continue to enclose it even when of large size.
2. Generally the entire glandular portion of the organ is replaced by new growth, but sometimes the atrophied deep brownish glandular portion may remain under considerable compression at the peripheral part.
3. The tumor consists of nodules, which vary considerably in size and are generally surrounded and separated by strands of fibrous tissue in varying amount.
4. On section the cut surface of the tumor parenchyma is of medullary yellowish gray-white colour and of soft or somewhat firm consistence, whilst here and there creamy yellowish red-gray islets which represent the softened necrotic areas, and various-sized irregular deep red hemorrhages can be seen, so that these figures give an almost marble-like appearance.
5. The spermatic cord and the inguinal and lumbar lymph-nodes are frequently apt to become involved, and distant metastases perhaps may occur (dissemination in the peritoneal cavity).

Microscopical examination

1. Corresponding with the macroscopic appearance, bands of fibrous tissue are seen traversing the section in all directions. These vary in width and divide the parenchyma into islets of various sizes and shapes, giving an alveolar appearance.

2. The tumor cells are roughly polyhedral, spheroidal, or ellipsoidal in shape, the cytoplasm stains in general feebly with eosin and appears homogeneous or slightly granular, varying in size from 11 μ to 35 μ in diameter.

The nuclei are of three kinds, according to their shape, size, and staining qualities.

(i) Spheroidal or short ellipsoidal, deeply stained small nuclei with invisible nucleoli.

(ii) Ellipsoidal large nuclei with vacuolar appearance, in which only the nuclear membrane and nucleolus have taken on the stain, and the latter stains bright red with eosin (usually only one, sometimes two or three in number).

(iii) Nuclei which show intermediate characters between (i) and (ii).

The size of these nuclei varies from 6.5–16 μ in diameter. Multinuclear cells with strikingly large cytoplasm and a circular arrangement of their nuclei about a central lumen are numerous in all cases. Mitotic figures are abundant in the tumor cells, most of them being bipolar, though figures with three, four, or more poles, and cells with irregular distribution of chromosomes are not infrequently met with. Generally the tumor cells are rich in glycogen, but only a small amount of fat is to be seen; there are small areas of haemorrhage and necrosis in the tumor parenchyma, and the fat can be shown circumscribed especially in the latter, in sections stained with Sudan III.

3. Traversing the section in all directions and cutting up the parenchyma into islets, the fibrous stroma gives off finer strands—sometimes only a few fibers—running in towards the peripheral portion of the enclosed parenchyma from the main circumferential band; but the tumor cells lie along the walls of the alveoli without intimate connection with them and are packed together without fibrillar intercellular substance, so that not all the tumor cells are intermingled with the fibrous stroma. The stroma represents more or less round-cell infiltration, which mostly consists of lymphocytes; and reactive proliferation of the connective tissues may sometimes be seen. Elastic fibers appear generally in small amount.

4. In the atrophied residual glandular portion, spermatogenesis is usually not visible; only one case among four with surviving glandular tissue showed spermatogeny. The cells in the atrophic seminiferous tubules greatly resemble tumor cells morphologically. Interstitial tissue and membrana propria of the seminiferous tubules are in general

increased, and masses of granular cells in the interstitium (*Zwischenzellen*) appear atrophic.

As compared with these observations in Japan, Sticker (6), in his compilation of the European literature on the occurrence of tumors in the lower mammals, reports that of 298 malignant tumors in horses, but 11 were in the testicle; in cattle, of 110 tumors none were in the testicle, and there were none among the recorded neoplasms of sheep (7 cases), of cats (7 cases), or of swine (7 cases). But of 766 tumors in dogs, 18 were in the testicle. Of 305 cases of bovine tumor observed in the slaughter house at Glasgow, according to Trotter (7), there was none in the testicle. Undoubtedly these figures give an entirely false impression as to the frequency of testicular tumors in the lower animals, since so few old, uncastrated horses, cattle, swine, or sheep come to the slaughter houses, where most of the neoplasms are detected. According to Caspar (4) a testicular cancer has been observed in a cat by Leiserling, metastases having been found in the mesentery of the transverse colon. A case of carcinoma of the bovine testicle is mentioned without details by Murray (8), and Williams (9) says that in the ass sarcoma of the testicle has been described; he also mentions the occurrence of tumors in the testis of swine, but without citing the origin of these reports. Wolff (10), in his compilation of the literature, refers to several cases of testicular tumor in dogs and horses, but records none in mice or other animals. He refers to the case, reported by Axe, of a tumor arising in an ectopic testicle in a pony, and several other cases in the literature suggest that in dogs and horses, as in man, the ectopic testicle is especially likely to undergo malignant transformation.

One of us (H. G. W.) has observed two cases of primary tumor of the testicle in dogs, which, because of the infrequency of recorded cases in the literature, are briefly described here, as follows:

Case I. A pure blooded Pomeranian, age five and one-half years, was brought to the laboratory by Dr. J. W. Walker, in order that a growth involving the right testicle might be removed by operation.

This growth had been noticed at least two years before and had grown very slowly. The operation was done under anesthesia; the animal recovered and is now in apparently perfect health three years after the operation. No metastases could be palpated in the groin or elsewhere. The animal had been suffering from extensive loss of hair for some time before the operation, but after it the normal coat was restored. The growth was about 3 x 2 x 2 cm., involving the body of the testicle as an encapsulated nodule and leaving a compressed remnant of testicle tissue at one side. There was no infiltration, and the epididymis was not involved.

Microscopically it shows irregular coarse lobules with heavy fibrous tissue trabeculae, packed full of large cells with little cytoplasm and pale vesicular nucleus closely resembling the spermatogonia. The capsule is very dense and contains a few islands of tissue cells. Despite the heavy fibrous tissue the cells do not seem to be compressed. In the residual testicle tissue there is some spermatogenesis going on. This seems to represent an unusually benign, slow-growing type of the usual large cell tumor of the testicle.

Case II. A mongrel fox terrier dog, apparently moderately old, was killed in the course of experimental work in the Department of Physiology of the University of Chicago, and an autopsy was performed by Dr. A. B. Luckhardt. The right testicle, which was within the scrotum, was replaced by a tumor about 6 cm. in diameter, entirely contained within the tunica albuginea, and not adherent to the surrounding tissues. No metastases could be found in the regional nodes or elsewhere. The prostate, however, was swollen, and irregular in shape and consistence, and contained a purulent fluid. The left testicle lay within the inguinal canal and was decreased in size to about one-half the normal dimensions, although the epididymis was apparently normal. Nothing is known of the history of this animal, but it was striking in that the teats resembled those of a bitch that has nursed repeatedly.

Microscopically the tumor resembles the usual type of alveolar carcinoma seen in human cases (fig. 1). The cells are large, consisting chiefly of a large vesicular nucleus; the stroma is heavy and coarse, and divides the tumor not only into alveoli but also into lobules; only in a few areas is it cicatricial. Mitoses are abundant. A striking peculiarity in this tumor is the tendency of the cells in some alveoli to form long cords parallel to each other and at right angles to the basement membrane, producing an unusual effect of palisade arrangement.

Sections through many parts of the tumor show no residue of testicle tissue, and no teratomatous elements. The prostate shows an extensive round cell interstitial infiltration, with numerous polymorphonuclear leucocytes within the tubules. The atrophied ectopic testicle shows an epididymis resembling that of the preadolescent testicle, while the testicle itself consists of ill-defined tubules with ordinarily but a single row of irregular cells near a thickened basement membrane, although the center of the tubules contains much protoplasmic debris which is

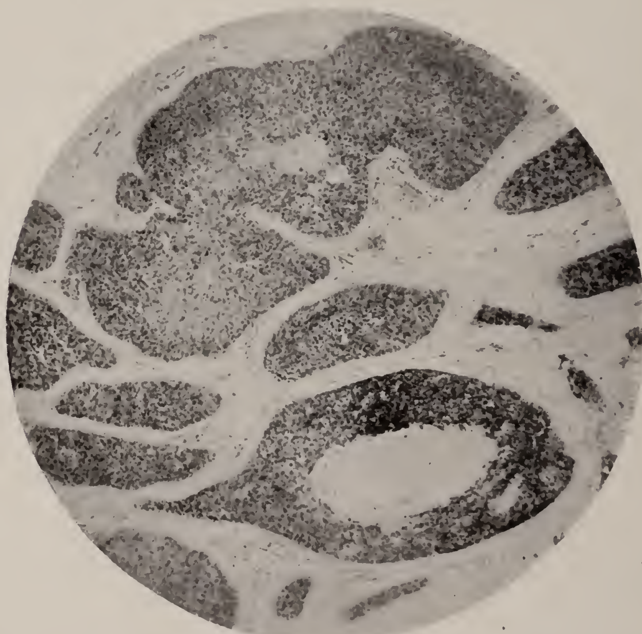


Fig. 1. Primary Carcinoma of the Testicle of a Dog. (× 80)

often pigmented. The stroma is moderately increased, but Leydig's cells are not conspicuous. Several of the tubules are full of large deeply staining cells resembling those of the tumor of the opposite testicle. These give the impression that in these isolated atrophic tubules neoplastic proliferation is beginning to arise independently.

In other species of animals, although castration is not commonly practiced, testicle tumors are rare. The reviews of the literature on spontaneous tumors of birds and fowls by Joest

and Ernesti (11), and by Pentimalli (12), mention only three testicular tumors. One was a teratoma replacing the testicle of a chicken, described by Winokuroff (13) and the other two were cases of sarcoma of the testicle in parrakeets (*Palaenoris Eupatrius?*), reported by Fox (14). One of these was bilaterally symmetrical, probably derived from the testicle, and described as a large round cell alveolar sarcoma; the other was unilateral, and undoubtedly arose in the testicle. In his extensive autopsy experience with wild animals Fox has found no case of tumor of the testicle, nor could we find any such cases in the literature, unless we include the instance recorded by Pick and Poll (15) of a cystoma testis with carcinomatous areas and a secondary nodule, which occurred in the testicle of a Japanese giant salamander (*Kryptobranchus japonicus*).

From such descriptions of the histological findings of the testicular tumors of lower animals as are found in the literature, and which are mostly meagre, these growths seem to present the same characteristics as those observed in man. The prevailing type is those tumors of large polyhedral cells in alveolar arrangement, which are often described as alveolar sarcoma or, when the normal relation of the tumor cells to the nutritive vessels is conspicuous, angiosarcoma. Lymphoid infiltration of the stroma is also commonly described in animals as well as in human tumors. Ewing (16), in his critical review of testicular neoplasms, says of the human material: "The commonest tumor of the testis is an embryonal carcinoma, alveolar or diffuse, with polyhedral or rounded cells and often with lymphoid stroma." This statement holds equally well for the testicular growths of animals as described in the literature. Ewing also states: "These tumors are probably one sided developments of teratomata," an origin which is not suggested by those authors who describe animal tumors, although Caspar (4) quotes Kitt as saying that chondromas are found repeatedly in the testes of horses; on the other hand, Kimura does not even mention the occurrence in horses of tumors suggesting a teratomatous character. Furthermore, Frank (17), in his discussion of the histogenetic origin of testicle tumors, concludes that the typical

large cell tumors are not derived from teratoid tumors, but from spermatogonia. Kimura, in his study of equine material, also came to the conclusion that these tumors are derived from the epithelium of the seminiferous tubules.

TESTICULAR TUMORS IN MICE

In 19,000 autopsies on mice of the Slye stock we have found 28 tumors arising in the testicle. These figures, it should be emphasized, apply to mice living a natural life and reaching as great an age as it has been possible to make then attain by the best of care and the most rigorous hygienic precautions; all died natural deaths, without having undergone any experimental interventions or manipulation. Of the 19,000 about one-half were males. Statistics have not yet been compiled as to the total number of tumors arising in male mice, although they are much less common than in females, most abundant being tumors of the lung, subcutaneous tumors, sarcomas, tumors of the testis, and adenoma of the liver. As we have pointed out in previous papers, the great predominance of females among tumor mice, observed and emphasized by other writers, depends solely upon the frequency of mammary tumors. Excluding these and other sex gland tumors, we find an approximately equal distribution. Thus, in our published lung tumor series (18) of 160 cases there were 42.6 per cent in males, and 57.4 per cent in females; in 28 liver tumors (19) there were 50 per cent in each sex; in 8 cases of cancer of the stomach (20) also, equal numbers were observed in each sex. Sarcomas are more abundant in female mice than in males in the ratio of 2 to 1 because the mammary gland is a common site of sarcomas (21). The tumors of the testicle offset in large measure the tumors arising in the ovary.

That we have obtained so large a number of tumors of the testicle is explained by the fact that all these tumors, with one exception (7308), occurred in the mice in a single strain, No. 90, and its hybrid derivatives. This exceptional case was not a typical testicular growth, but a spindle cell sarcoma. As has

been emphasized in previous papers, not only the tendency to develop cancer depends on the ancestry of the mice, but also the localization of tumors in special organs or tissues (see Slye (22)). Had not this particular strain been developed, hybridized extensively, and followed for several years, there would have been but a single case of testicle tumor to record, and that a sarcoma. We may mention the fact that some forms of growth observed in other laboratories seem to be relatively infrequent in the Slye stock, e.g., the keratinizing type of lung tumors described by Tyzzer and Haaland. and the preputial gland adenomas observed especially in Bashford's laboratory. As material accumulates this tendency of certain tumors to occur in certain strains of mice becomes more and more distinct; this has its counterpart in human pathology—e.g., the uterine fibroids of the negro, and the repeatedly described predominance of tumors of certain viscera in certain families.

Most of these new growths of the testis are essentially benign in character, developing very slowly, rarely ulcerating, generally distinctly limited by the tunica albuginea, and in no case with distinct remote metastases (fig. 2). Some cases have remained under observation for as long as eight months, and in only a few instances have they seemed to be the cause of death, through urinary retention or ascending suppurative nephritis (5 cases), local necrosis and suppuration (3 cases), or hemorrhage (3 cases). They have varied in size from about 5 mm. in diameter to one 30 by 23 by 23 mm., the larger tumors usually showing much necrosis, although not often infected because of the protection afforded by the intact tunica albuginea. Contrary to the experience with other species, no case has been observed in an ectopic testicle, a malformation that seems to be rare in mice. As with other tumors, these growths do not appear until the mice are of middle age or older; the youngest animal in this series was 9 months old when the tumor was first seen and died when 14 months old; the oldest was $3\frac{1}{2}$ years at death, the tumor having been under observation for eight months.

Four tumors seemed to result directly from trauma, appearing in mice while under observation because of injuries to the genital

organs received in fighting. One of these was a typical spindle cell sarcoma (3117) which arose at the site of a bitten wound of the testicle, while simultaneously a similar sarcoma arose in a

Fig. 2. Primary Tumor of the Right Testicle of a Mouse, Showing the Gross Appearance Usual in These Cases

The lower pole of the tumor is necrotic. It is entirely encapsulated and there are no metastases. Microscopically this was a typical "mesothelioma" or "orchidoblastoma" as shown in figures 3 and 5.

wound on the back. Of the other three, two were of the usual large cell type (3561 and 8145) while one (585) again was a spindle cell growth. It is, of course, impossible to tell how many of the other 24 tumors followed trauma.

Several instances of simple cyst formation, probably secondary to trauma, have not been included in this series.

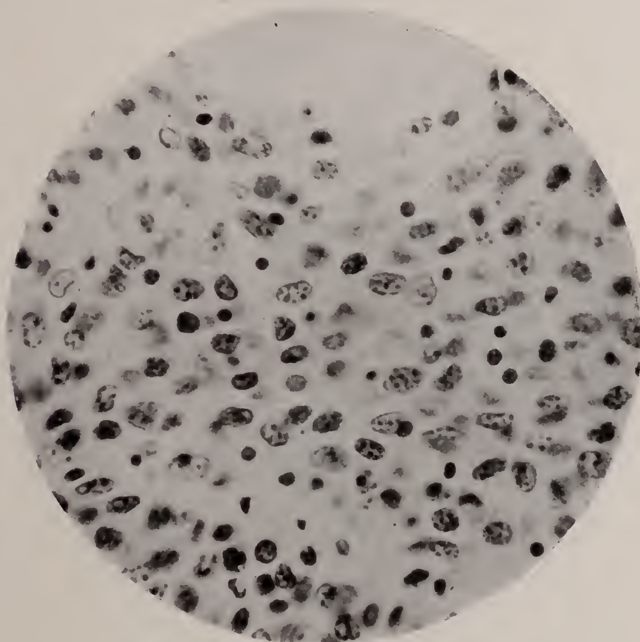


Fig. 3. Showing the Typical Cytological Characters of Most of the Tumors of this Series. (× 480)

At the lower edge of the field is a blood-space, showing the direct relation of blood and tumor cells, without distinguishable vessel wall.

Microscopically the great majority of these tumors are all of one structure (fig. 3), which is entirely typical and characteristic. These typical growths are composed of large rather pale and delicately architectured cells. The nuclei are vesicular, often markedly so, with the chromatin in coarse granules and not uniformly arranged. There is an abundant cytoplasm, staining faintly, usually with well defined margins and of polygonal

outline unless compressed into flattened or spindle shaped cells (fig. 4). Mitotic figures are fairly abundant in some specimens, but never extremely numerous; pycnosis and karyorrhexis are frequently seen. Commonly there is a tendency for the cells to be arranged in cords, as in liver tissue, and an alveolar structure is often distinct although never very well developed. As with all mouse tumors, the stroma is seldom so dense as in

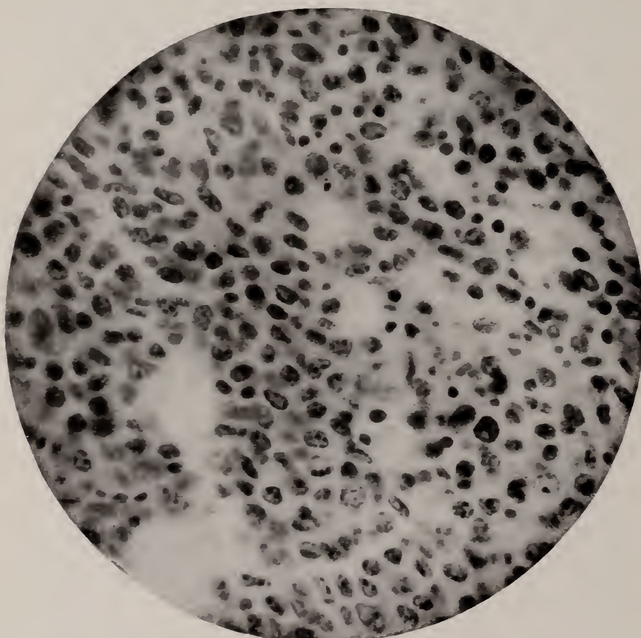


FIG. 4. TUMOR CELLS SOMEWHAT COMPRESSED INTO A MORE SPINDLE CELL TYPE. (× 480)

corresponding human tumors. Usually the tumor is extremely vascular, with large blood channels (fig. 5), the walls of which are often composed solely of tumor cells, although sometimes the vessels have definite fibrous walls; hemorrhages are frequent, and in many sections old pigment and cholesterol masses indicate the site of former hemorrhages. Cells with atypical and giant nuclei are common, and multinucleated cells are often found. Sometimes tubule-like structures are present, but these cannot

be identified as typical "rosettes" such as have been described in human testicular neoplasms. Multipolar and other abnormal forms of mitosis have occasionaly been seen.

Necrosis is present to greater or less degree in most of the specimens, and in some nearly all the tumor is necrotic, as if strangulated by the dense capsule. Often the necrosis is uniformly distributed in those cells more remote from the blood

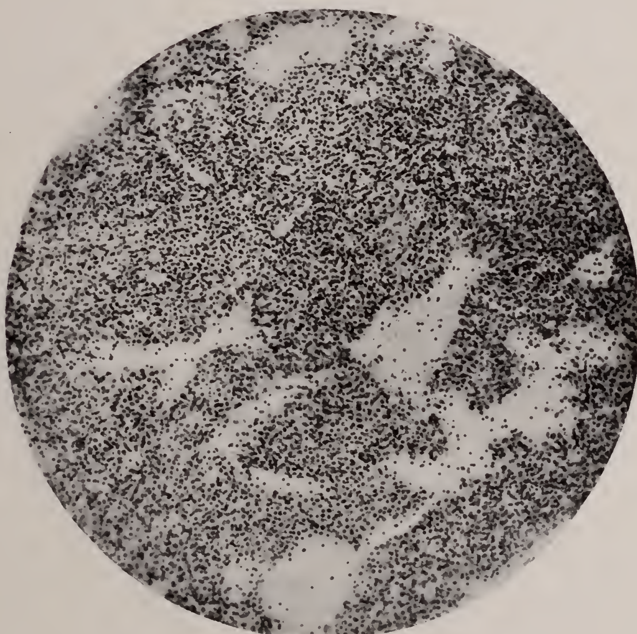


FIG. 5. SHOWING THE GENERAL HISTOLOGICAL FEATURES CHARACTERISTIC OF MOST OF THE TUMORS OF THE MOUSE TESTIS. (× 110)

The intimate relation of tumor cells to blood-spaces is a striking feature.

spaces, leaving about each vessel a zone of living tumor cells so that the resulting picture resembles a "hemangiosarcoma," often quite characteristically (fig. 6). This recalls the fact that a common diagnosis of testicular tumors in man is "angiosarcoma."

In every case the original testicle tissue has been almost or quite completely destroyed, so that only occasionally can the

remains of a single isolated seminiferous tubule be found. Most often the tubules are only indicated by a partly calcified tubular outline, identified solely by the sperm heads which persist in the lumen. If more tubules remain they are generally compressed against the capsule, often heavily pigmented. The capsule of the testicle always persists, and although usually invaded, it is perforated only in a few instances; occasionally the capsule

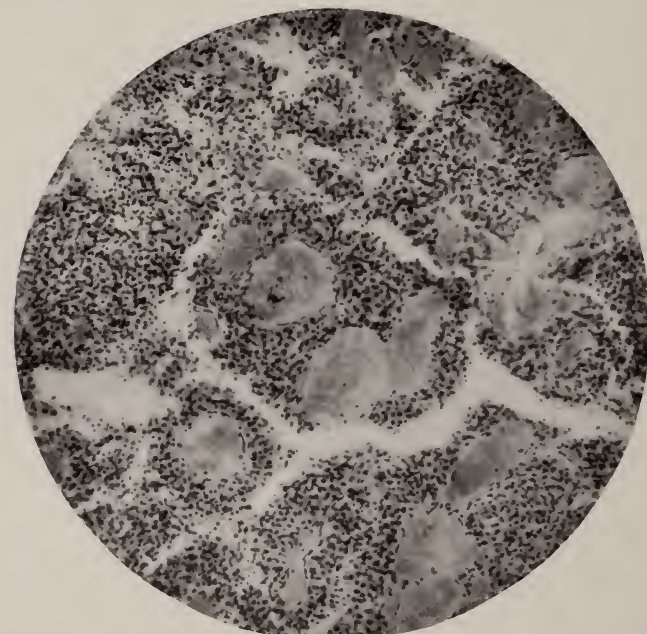


Fig. 6. Testicular Tumor in Which the Tumor Cells are Generally Grouped About Blood-Spaces, Thus Resembling "Angiosarcoma." (× 110)

contains what seem to be lymph-vessels filled with plugs of tumor cells. When seminiferous tubules remain for comparison, it is usually apparent that the typical tumor cells have much the same structure as some of the cells of the tubule, the spermatogonia.

The stroma is usually scanty and very delicate, so that large areas often show no evidence whatever of stroma elements.

When best defined it divides the tumor cells into indistinct alveoli, and frequently shows an abundance of small lymphoid cells. In many instances clear cyst-like spaces are found without any distinct lining, but giving the impression of resulting from softening and absorption of the tumor, rather than from cyst formation. Blood pigment is often found in phagocytic cells in the stroma and capsule.

Five of the tumors present a histological structure resembling sarcoma, but in three of these, at least, it is probable that the sarcomatous appearance is merely the result of flattening of the cells by pressure. This assumption is supported by the finding, in some of the sarcoma-like tumors, of cell areas resembling the more usual type of testicle tumors, and by the occasional presence, in the ordinary tumors, of areas of spindle cells that are undoubtedly the result of local pressure, e.g., beneath the capsule. These tumors would seem to correspond to the type of tumor often found in the human testicle and commonly diagnosed as alveolar sarcoma or angiosarcoma. One of them (10062), however, presents so much finely divided intracellular golden pigment, without evidence of old hemorrhage as a source of this pigment, that it may possibly represent a tumor derived from the interstitial cells of Leydig, for tumors of such origin have been described in man.

Of the remaining two, one is a typical spindle cell sarcoma which has been described in our paper on sarcoma in mice (case 3117). This mouse was bitten on the back and on the genitals. From each of these sites arose a typical spindle cell sarcoma. The genital tumor seemed to arise from and replace the testicle. Both neoplasms developed at the same time, and seemed to be independent, primary, spontaneous tumors. This mouse also had an adenoma of the liver. The fifth tumor (fig. 7) also seems to be a typical spindle cell sarcoma (7308), in which no persisting elements of testicle can be found; at one point it has grown through the capsule, so that the growth is bilobed. Mallory's connective tissue stain supports the interpretation of the growth as a true sarcoma, the individual cells lying in and evidently producing a matrix of collagenous fibers.

This tumor is especially interesting as being the only growth occurring in an animal not derived from strain 90.

It is a matter for comment that we have found no example of the teratoid type of tumor that is so common in the human testicle. In not a single section have we found a trace of heterologous tissue elements that might suggest a teratomatous origin. Twice we have found simple cysts, apparently resulting

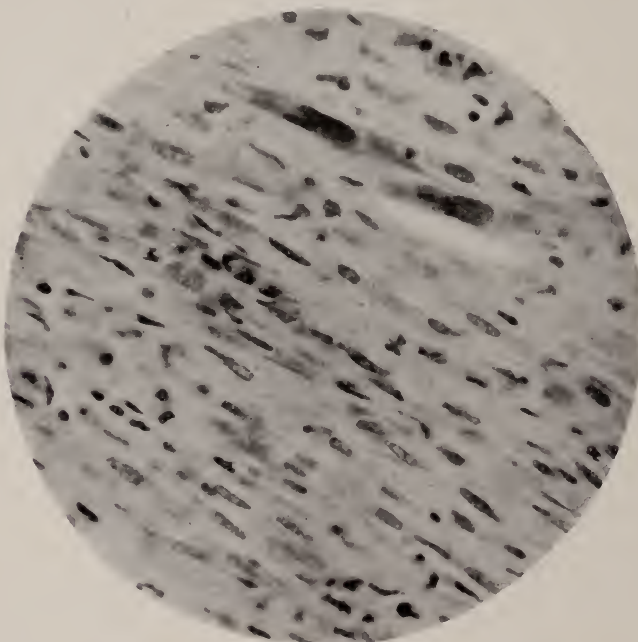


Fig. 7. Spindle Cell Sarcoma Arising Within the Testicle of a Mouse. (× 480)

from traumatism and subsequent absorption of the testicle elements. Indeed, in these 19,000 autopsies which have furnished so rich a tumor material, we have found but a single instance of true teratoma, and this arose in the ovary. We have made complete serial sections of three of these testicular tumors, without finding any teratomatous elements. This evidence would seem to us to indicate that, at least in the mouse, the orchidoblastomas do not ordinarily arise in teratomas, which have been

maintained by some to be the common origin of the similar tumors in man. The histological evidence of the similarity of the tumor cells to the spermatogonia would seem to support Frank's view that these neoplasms arise from the epithelium of the seminiferous tubules.

In view of the extremely heterotypical structure of these growths, the total absence of metastases in this series is surprising, especially as metastasis is so extremely extensive and early in the structurally similar tumors of man. And in other mammals, also, metastases are described from testicular tumors, especially in the horse.

One case alone (9441) showed involvement of both testicles, the structure of each growth being the same. In our first canine case, however, the animal showed no recurrence three years after orchidectomy, and the second had developed no visible metastases when autopsied. Several of our sections of mouse tumors show what appear to be plugs of tumor cells in the lymph-channels, while the blood-spaces are commonly lined with naked tumor cells, apparently offering every opportunity for extensive metastasis. Nevertheless, close inspection of the lung sections has failed to show even the retrogressing tumor cell emboli which are so often found in the lung in connection with abdominal carcinoma, as pointed out by M. B. Schmidt.

In but five cases was the tunica albuginea definitely perforated by macroscopic growths. One of these, however, showed very extensive formation of secondary nodules (7870), there being several large tumors outside the testicle (fig. 8). Here the growth consisted of six contiguous nodules, each distinct and enclosed in a capsule. Histologically all these nodules are of similar structure, characterized by an unusually large amount of lipoid material and many necrotic areas. So far as structure is concerned this tumor does not seem to be more malignant than other tumors that do not show the multiple nodules. In one case (2753) the growth extended along the spermatic cord, so that the mass resembled a hernia.

As noted in previous studies of special types of tumors in mice, animals with one tumor are likely to exhibit a growth of

some other sort, apparently more often than would be explained by the law of probability. With testicular tumors the coexistence of other neoplasms would not be expected to be so

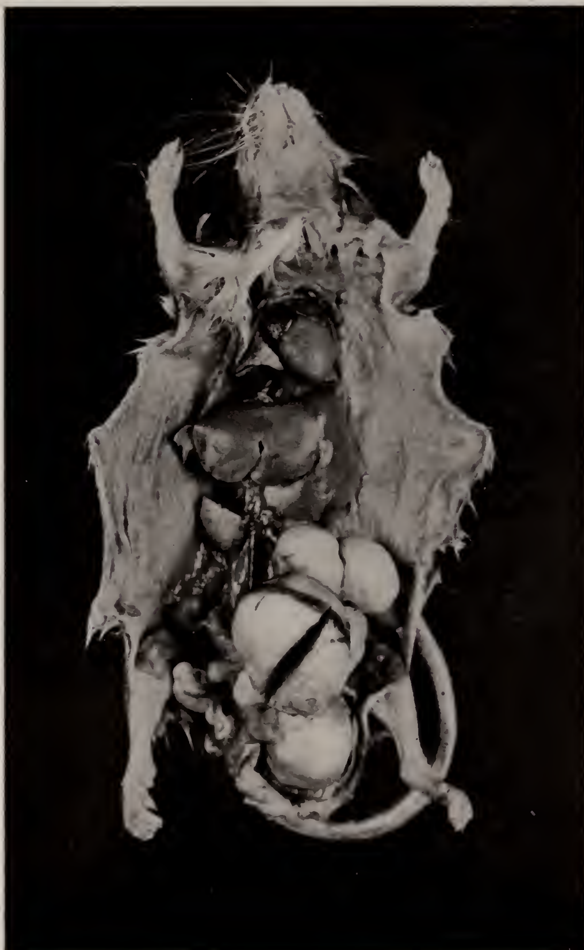


FIG. 8. TUMOR OF THE LEFT TESTICLE WITH THE PRODUCTION OF SEVERAL LOCAL METASTATIC GROWTHS, BUT NO REMOTE METASTASIS

frequent as was observed with the tumors previously described (lung tumors, liver tumors, sarcomas) because the commonest of all tumors in mice, the mammary carcinoma, does not often

occur in males. Nevertheless, among our 28 cases of tumor of the testis we find the following seven instances of multiple tumor formation: One mouse with a subcutaneous spindle cell sarcoma and an adenoma of the liver; three with papillary adenomatous growths arising in the lung; two with osteosarcomas, one arising in the thigh (16370), the other in the subcutaneous tissues near the fore leg (8745). In still another mouse there was a subcutaneous growth in the neck of such a character that a diagnosis has not been made (5037). This growth was about 1 cm. in diameter and 2 to 3 mm. thick, consisting of a mass of cells with strongly basophilic cytoplasm, of a finely granular, "ground glass" character, resembling that of plasma cells. The nuclei, however, are centrally located and have not the characteristic chromatin arrangement of the plasma cell nucleus. The cell boundaries are distinct, and the cells tend to form long rows and strings, recalling the structures sometimes seen in the so-called "lymphangioma hypertrophicum." It is probable that this growth is a true neoplasm, but as long as its nature remains undecided its neoplastic character cannot be definitely determined. The character of the cells is such as to make it improbable that it is a metastatic growth. The occurrence of multiple primary new growths in no less than 25 per cent of these male tumor mice is striking evidence of the existence of a predisposition to tumor formation.

SARCOMA OF THE SEMINAL VESICLE

In the literature on tumors of the lower mammals we have found mention of but one instance of a neoplasm arising in the seminal vesicles. Such growths are also rare in man, Ceelen (23) in 1912 having been able to find reports of but five cases of carcinoma and one of sarcoma, to which he added a case of fibromyoma. We have not been able to find records of any other human or animal cases, except that described by Flexner and Jobling (24) as "originally regarded as a sarcoma; probably a teratoma, from which an adeno-carcinoma was developed." This tumor, which has been used extensively in transplantation,

was found in a white rat, as a tumor "the size of a walnut" attached to the left seminal vesicle, without production of metastases. It was first described as a polymorphous sarcoma, but with included glandular elements. In the course of transplantation the carcinomatous elements developed and replaced the sarcomatous structures, becoming established as an adeno-carcinoma by the twenty-eighth generation.

FIG. 9. PRIMARY SARCOMA OF THE SEMINAL VESICLE OF A MOUSE. (× 110)

Note the numerous giant cells, and the polymorphous character of the tumor cells.

A single case of primary tumor of the seminal vesicle has been observed in one of the mice examined in this laboratory (15443). This animal died of pneumonia, and autopsy disclosed the left seminal vesicle constricted at a point midway of its length, with the tip shrunken. The remainder of the organ is distended into a mass 10 by 8 by 8 mm., rather soft, but containing no fluid. The right seminal vesicle is similarly con-

stricted, but there is no tumor tissue and the tip is merely distended with yellow secretion; the rest is in shape like the left, but slightly smaller and yellow in color. There were no metastatic growths to be found. The testicles seem normal, and the kidneys show a slight nephritis. Microscopically the lumen of the right vesicle is found to be distended by a solid cellular growth, limited by the somewhat thickened peritoneal coat and the remains of the muscular coat, except at one point where these structures have been penetrated by the neoplasm. Only a few small groups of columnar epithelial cells remain to represent the original mucosa. The tumor is composed of spindle cells of varying size, but mostly larger than in the usual spindle cell sarcoma (fig. 9). There are also many multinucleated cells, and numerous enormous single cells with a single giant nucleus, sometimes undergoing asymmetric direct division. Thin-walled blood-channels and blood-spaces lined only by unmodified tumor cells are abundant. There are no hemorrhages or necrotic areas, but some cystic spaces full of plasma. This is a typical large polymorphous celled sarcoma. Nothing was found to indicate a teratomatous or carcinomatous character, such as was observed in the Flexner-Jobling rat tumor, yet the fact that theirs was at first diagnosed as a polymorphous cell sarcoma is suggestive. In our case no transplantation experiments were attempted.

SUMMARY

Among 19,000 mice dying natural deaths and examined post mortem, about one-half of which were males, 28 instances of primary tumor of the testicle were found. Most of these resembled in all essential features the tumors that arise in the testicle of man and other animals, consisting of cells closely resembling the epithelium of the seminiferous tubules, arranged in an alveolar structure. Despite great vascularity and a markedly atypical structure, no remote metastasis was observed, although in one case a series of six contiguous independent nodules was formed, and one case showed bilateral testicular tumors. Two of the growths seemed to be true spindle cell sarcomas, one arising at the site of a wound. Three of the typical "or-

chidoblastomas" also followed trauma. No evidence could be obtained that any of these tumors had arisen in a teratomatous growth, and no cases of teratoma have been observed.

One case of polymorphous cell sarcoma of the seminal vesicle of a mouse is described, apparently the second case of a tumor of this organ reported in a lower animal.

Two cases of primary spontaneous tumor of the testicle in dogs are described.

With the exception of one sarcoma, all the 28 neoplasms of the mouse testis occurred in the members of a single strain of mice and its hybrid derivatives, thus substantiating the statement that heredity influences the incidence of tumor development in different organs or tissues. This fact also probably explains the absence of any recorded cases of tumor of the testis in mice from other laboratories.

REFERENCES

(1) McCoy: Jour. Med. Research, 1909, N.S., xvi, 285.
(2) Woolley and Wherry: Jour. Med. Research, 1911, N.S., xx, 205.
(3) Beatti: Semana med., 1917, xxiv, 643.
(4) Caspar: Lubarsch-Ostertag, Ergebn. d. allg. Path., 1896, iii², 799.
(5) Kimura: Gann, 1917, xi, 38.
(6) Sticker: Arch. f. klin. Chir. (Langenbeck), 1902, lxv, 616.
(7) Trotter: Jour. Comp. Path. and Therap., 1911, xxiv, 1.
(8) Murray: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, p. 41.
(9) Williams: The Natural History of Cancer, p. 96.
(10) Wolff: Die Lehre v. d. Krebskrankh., 1913, iii¹, 217.
(11) Joest and Ernesti: Ztschr. f. Krebsforsch., 1915, xv, 1.
(12) Pentimalli: Ztschr. f. Krebsforsch, 1915, xv, 111.
(13) Winokuroff: Inaug. Diss., Bonn, 1908; cited by Joest and Ernesti.
(14) Fox: Jour. Path. and Bacteriol., 1912, xvii, 217.
(15) Pick and Poll: Berl. klin. Wchnschr., 1903, xl, 572.
(16) Ewing: Surg., Gynec., and Obst., 1911, xii, 230.
(17) Frank: Frankfurt. Ztschr. f. Path., 1911, ix, 206.
(18) Slye, Holmes, and Wells: Jour. Med. Research, 1914, N.S., xxv, 417.
(19) Slye, Holmes, and Wells: Jour. Med. Research, 1915, N.S., xxviii, 171.
(20) Slye, Holmes, and Wells: Jour. Cancer Research, 1917, ii, 401.
(21) Slye, Holmes, and Wells: Jour. Cancer Research, 1917, ii, 1.
(22) Slye: Jour. Cancer Research, 1916, i, 479.
(23) Ceelen: Virchows Arch., 1912, ccvii, 200.
(24) Flexner and Jobling: Monographs of the Rockefeller Institute, 1910, No. 1; Jour. Amer. Med. Assn., 1907, xlviii, 420; Centralbl. f. allg. Path. u. path. Anat., 1907, xviii, 257.

CANCER IN HAINAN, CHINA

A PRELIMINARY STATISTICAL STUDY OF 131 OPERATIONS WITH SPECIAL REFERENCE TO AGE INCIDENCE, ANATOMICAL DISTRIBUTION, AND ETIOLOGY

NATHANIEL BERCOVITZ

Kachek, Hainan, China

Received for publication, June 20, 1918

Cancer is regarded as a disease of the later decades of life. Its etiology is still obscure, although many contributing causes such as continued local irritation of a chemical, mechanical, or inflammatory nature have been held responsible. Indeed, an almost innumerable list of possible factors, including bacteria and protozoa, has been studied with reference to etiology, but cancer still remains withal a problem unsolved.

The disease, as observed by the author in Hainan, presents a number of phases which differ somewhat from those usually encountered. These phases include the early age incidence of the disease, the unusual anatomical distribution, and a difference in the causes commonly considered as predisposing.

In the study of these statistics the medical conditions peculiar to China must be remembered, viz., the gradual turning of the people to western medicine, the fact that many cases which come to the hospital are inoperable and come only after all native remedies have failed, and, finally, the fact that until very recently prejudice has kept the women of China away from medical and surgical aid. For these reasons it may be assumed that the statistics of the next few years will differ somewhat from the present figures.

The cases were operated upon by the author in Hoi How and Kachek, and by members of the staff in the Hoi How Hospital. Lack of facilities prevented microscopic examination, to the great regret of the writer.

AGE INCIDENCE

Table 1 shows the proportion of cases seen in the early decades of life. This fact is mentioned without comment, except to say that the percentage is unusually high.

TABLE 1

Total number of cases, 131

AGE	NUMBER OF CASES	PER CENT
Above 40 years	65	49.7
Under 40 years	66	50.3
	131	100.0
Under 35 years	47	36.0
Under 30 years	38	29.0
Under 25 years	25	19.0

There are no known etiological factors which would account for 50.3 per cent of the cases occurring before the age of forty.

ANATOMICAL DISTRIBUTION

Table 2 groups the cases roughly according to anatomical location, with reference to the age incidence and sex.

TABLE 2

ANATOMICAL DISTRIBUTION	NUMBER OF CASES	PER CENT	AGE		MALE	FEMALE
			Above 40	Under 40		
Penis	29	22.1	18	11		
Skin	29	22.1	16	13	23	6
Neck	26	19.8	8	18	22	4
Face; Scalp	21	16.0	9	12	17	4
Breast	10	7.6	7	3	2	8
Bone	8	6.1	3	5	6	2
Testicle	3	2.4	2	1		
Anus	3	2.4	1	2	2	1
Tongue	1	0.7	1	0	1	0
Colon	1	0.7	0	1	1	0

The most noticeable feature of table 2 is the high percentage of carcinoma of the penis. Except for the high incidence of syphilis among the Chinese male population and attempts to cure the primary sore by the application of highly caustic native remedies, no reason can be assigned for this large figure.

Under the heading of "skin" are grouped all the cancers of the skin covering the extremities and the trunk. These are often encountered, perhaps because ulcers of the arms and legs are very common in China. The ulcers are treated, not by cleansing and the application of healing remedies, but by the crude native methods of applying plasters, either of mud mixed with cow dung, or of leaves enclosing irritating substances. As a result, they eat deeply into the flesh and heal—if they heal at all—with contractures of the muscle sheaths and consequent deformity; or they produce an exuberance of scar tissue with the formation of keloids; or they degenerate into malignant growths.

Counter irritation is extensively practiced by the Chinese with the idea of driving out the evil spirits supposed to be the cause of disease. The counter irritation takes many forms, one of which is a piercing of the skin. The resulting wound is always infected, and in healing may degenerate into a malignant growth.

Malignant disease of the cervical glands is frequently seen. The table shows that 26 of the 131 cases belong to this class, and in the experience of the author most of the inoperable casesof cancer met with in Hainan have been of the neck. The etiology in these cases is exceedingly obscure, as the great proportion of cases occur in the period under forty years of age, one case being only four years of age. In most of the cases there are no marks on the neck to indicate previous counter irritation, and infection from the throat might well be considered a contributing factor, especially in the absence of any other signs.

Cancer of the face and scalp includes epithelioma of the lip, malignant disease of the nose, and cancer of the skin of the face and scalp. A large proportion of these cases occur in the early decades of life, and aside from the considerations discussed in

cases of cancer of the skin it might be well to mention the fact that most of these people are much exposed to the rays of the tropical sun. This exposure has been regarded as a cause of rodent ulcer.

When the cases of skin cancer are grouped with those of the face and scalp, a total of 50 cases, or 38.1 per cent of the whole number reported, fall under this group; which, with carcinoma of the penis and of the glands of the neck make a total of 105 cases, or about 80 per cent of all the cases.

This leaves for consideration the following:

Cancers of the breast, 70 per cent of which are seen after the fourth decade. The number reported is only 10; but it must be remembered that the traditions of centuries have kept women from seeking aid from male physicians. In view of the changing conditions, the next few years should add greatly to our knowledge of this disease in China.

Eight cases of osteo-sarcoma present no unusual features. Three cases of carcinoma of the testicle, three of epithelioma of the anus, and one case of carcinoma of the descending colon are not unlike similar cases found elsewhere.

Two locations, so common in America and many other countries, are strikingly conspicuous by their absence from this list, viz:—the stomach and the uterus. Here again the existing conditions in China must be taken into consideration. The clinical diagnosis of cancer has occasionally been made, but not frequently, and only recently have abdominal operations of any kind been undertaken. Hence the physicians may not see the cases, especially as the Chinese like to treat such patients themselves. Or there may actually be a smaller number of cases because certain contributing factors are absent. Thus, while they do eat a little meat and a few vegetables, the diet of the Chinese is chiefly rice, and excesses in diet can not be afforded by the masses, who are very poor.

Ulcer of the stomach is rather infrequent. And as it has been shown by Rosenow and others that gastric ulcer is the result of streptococcic infection, it will be of further interest to know that scarlet fever and diphtheria are not known in Hainan, and

that appendicitis and acute articular rheumatism (non-gonorrheic) are only infrequently met with. In the absence of much gastric ulcer, therefore, the cases of cancer of the stomach might be expected to be few.

It is also of interest to note that the gastric crises of tabes dorsalis and general paresis are very rarely seen, notwithstanding the widespread incidence of syphilis and its very destructive tertiary lesions.

As to cancer of the uterus, the author has seen one case, which was inoperable. It is expected that more will be seen in the future, but it must be noted that uterine fibroids are rare; and notwithstanding the many cases of gonorrhea among men, cases of salpingitis and vaginitis seem to be very rare. Furthermore, stricture of the male urethra is not very common, and the question might very well be raised whether the gonococcus in Hainan may not be less virulent, because of the heat of the tropics. Still, this could hardly be the case, because it is as potent in its destructive influences on the eyes, the epididymis, and the joints as it is in America. On the other hand, are the cells lining the urethra, the tubes, uterus, and vagina less sensitive to the gonococcus in Hainan than in America? If inflammation of the female generative organs be considered a cause of malignancy, that cause, as far as is now known, seems to be largely lacking in Hainan.

Otherwise the lesions of the female generative organs are the same as in America, with the possible exception that malpositions are less frequent because of the more normal life and habits of the women here.

This brief statistical study of cancer in Hainan brings out the following points:

1. That cancer in Hainan is as much a disease of the early decades of life as the later, for which no reason can be assigned.

2. That cancer of the penis, and glands of the neck is unusually frequent; for the former an old primary sore may be the etiological factor; for the latter no reason can be assigned.

3. That cancer of the exposed surfaces of the body is very common. Inasmuch as in most cases these people wear a

scanty amount of clothing, the arms, trunk, and legs of men who work the fields being bare, the rays of the sun may be a contributing factor. But it is rather more likely that large ulcers, treated in the native fashion, and the sores resulting from counter irritation as practiced by the Chinese, are the etiological factors. This is all the more probable in view of the tendency to keloid formation and the universal infection of wounds.

4. Cancer of the stomach is infrequent. The absence of certain virulent streptococcic infections which are responsible for gastric ulcer may have something to do with this; or it may be that in this case, as in cancer of the uterus, the surgeon has not seen the cases.

5. Cancer of the uterus is infrequent. It may be that these cases have not been seen by the physician, but it seems rather suggestive that the virulent infections of the female generative organs seem to be infrequent here.

MORTALITY STATISTICS OF CANCER AMONG WAGE EARNERS: WITH OBSERVATIONS ON THE COMPARATIVE INCIDENCE OF THE DISEASE IN THE GENERAL POPULATION

LOUIS I. DUBLIN

Statistician, Metropolitan Life Insurance Company, New York

Received for publication, August 26, 1918

Although there have appeared in recent years many and important contributions to the mortality statistics of cancer, few of these have presented the facts in such detail as to show the incidence of this condition in the several age periods of life with the further distinction of sex and of color or race of the population. This is the merit of the data presented herewith.[1] They are to our knowledge original in the cancer literature and should well serve the nation-wide movement for the control of the disease. Many discussions which have centered around the cancer problem in recent years, such as the supposed increase of mortality and other questions, can be settled only as we know for a period of years the detailed facts of mortality for a relatively constant population. The present experience meets this requirement admirably and has the further merit that it reflects conditions in a large industrial group among whom, as will be shown later, cancer takes a heavy toll.

IMPROVEMENT OF CANCER MORTALITY STATISTICS

Special efforts were made in the course of the present inquiry to have the basic data as reliable as possible. Physicians certifying the causes of death often returned statements of cancer

[1] Being chapter ix of volume entitled: Mortality Statistics of Insured Wage Earners and their Families in the United States and Canada. Metropolitan Life Press, New York, 1919.

unqualified as to the organ or part affected, and in such instances, letters of inquiry were written and the physicians were asked to specify the type of tumor or cancer and the organs or parts first affected by the growth. The effect of this correspondence has been to increase the precision of the statistical results for cancer. While the data were not refined to the same point of completeness as characterized the recent investigation of the United States Bureau of the Census (1), the effort was made to cover fully the various parts and organs specified in the International List of Causes of Death.

CANCER ACCORDING TO ORGANS OR PARTS

During the six year period of this investigation, 37,666 cancer deaths were recorded at a rate of 70.0 per 100,000 persons exposed. Cancer was the sixth cause in order of numerical importance in this study. These deaths constituted 5.9 per cent of all the deaths in the experience. In the following table, the facts are arranged so as to show the number of deaths from cancer of the various organs or parts, and a few derivative ratios, including the death rates per 100,000 persons exposed are given:

TABLE 1

Mortality from cancer, specified according to organs or parts affected. Deaths, and death rates per 100,000 persons exposed. All color and sex groups combined. Experience of Metropolitan Life Insurance Company, Industrial Department, 1911 to 1916

Organ or Part Affected.	All Color and Sex Groups in Mortality Experience.			
	No. of Deaths.	Per Cent. of Total—All Causes.	Per Cent. of Total Cancer Deaths.	Death Rate per 100,000 Exposed.
CANCER—ALL FORMS	37,666	5.9	100.0	70.0
Cancer of the:				
Buccal cavity	1,353	.2	3.6	2.5
Stomach, liver	14,153	2.2	37.6	26.3
Peritoneum, intestines, rectum	4,482	.7	11.9	8.3
Female genital organs	7,882	1.2	20.9	14.7
Breast	3,579	.6	9.5	6.7
Skin	938	.2	2.5	1.7
Other organs, or of organs not specified	5,279	.8	14.0	9.8

Cancer and other malignant tumors of the stomach and liver constituted the largest single group of malignant growths, with 37.6 per cent of all cancers, at a rate of 26.3 per 100,000 persons exposed. Cancer of the female genital organs was next in importance, with 7882 deaths, constituting 20.9 per cent of all cancer deaths with a rate of 14.7 per one hundred thousand persons of both sexes. Cancers affecting the peritoneum, intestines, and rectum followed with 4,482 deaths, in all 11.9 per cent of all cancers at a rate of 8.3 per 100,000. These death rates, however, vary considerably with age and sex. In the following table, we give a comparison of the general cancer mortality experience for each of the main color and sex classes, showing separately the facts for cancer of the various organs or parts. The age data will be presented later.

TABLE 2

Mortality from cancer, classified according to organs or parts. Percentage of deaths, all causes, and death rate per 100,000 persons exposed. By color and by sex, 1911 to 1916. Experience of Metropolitan Life Insurance Company, Industrial Department, 1911 to 1916.

Organ or Part Affected.	Total Experience.		White Males.		White Females.		Colored Males.		Colored Females.	
	P. C. of Deaths—All Causes.	Death Rate per 100,000.	P. C. of Deaths—All Causes.	Death Rate per 100,000.	P. C. of Deaths—All Causes.	Death Rate per 100,000.	P. C. of Deaths—All Causes.	Death Rate per 100,000.	P. C. of Deaths—All Causes.	Death Rate per 100,000.
CANCER—ALL FORMS	5.9	70.0	4.3	50.4	8.5	88.4	1.8	31.0	5.2	87.8
Cancer of the:										
Buccal cavity	.2	2.5	.4	4.6	.1	.9	.1	2.5	.1	1.3
Stomach, liver	2.2	26.3	2.1	24.8	2.9	29.8	.9	16.2	1.1	18.5
Peritoneum, intestines, rectum	.7	8.3	.6	6.6	1.0	10.5	.2	3.4	.4	7.2
Female genital organs	1.2	14.7	—	—	2.4	25.3	—	—	2.2	37.9
Breast	.6	6.7	*	.1	1.1	11.7	*	.3	.9	14.7
Skin	.2	1.7	.2	2.2	.2	1.6	*	.8	*	.7
Other organs or of organs not specified	.8	9.8	1.0	12.0	.8	8.6	.4	7.9	.4	7.3

*Less than .05 per cent.

STATISTICS ACCORDING TO COLOR, SEX, AND AGE

We see from this table that for all ages, one and over combined, white persons show higher cancer death rates than colored persons, although the white female rate is only slightly higher

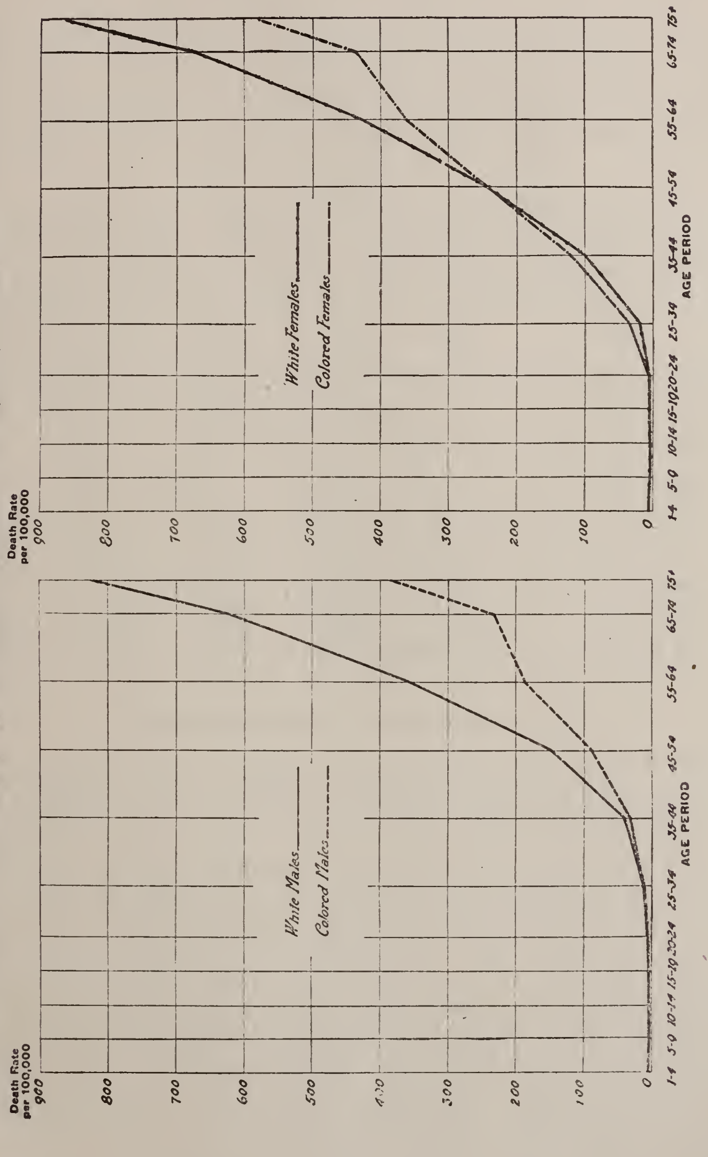
than that for colored females. Various differences between the cancer death rates of the color and sex classes occur for this disease as it affects various organs or parts. White males, for instance, show uniformly higher cancer death rates for each of the organs or parts, when compared with the rates for colored males. White females show significantly lower cancer death rates for this disease only as it affects the female genital organs and the breast. Cancer of the uterus and of the other genital organs shows a rate of 25.3 per one hundred thousand white females exposed and a rate of 37.9 for colored females. Cancer of the breast, in this present mortality experience, was recorded at a rate of 11.7 per one hundred thousand white females, and at a rate of 14.7 per one hundred thousand colored females. For the other chief organs or parts, cancer mortality of white females is greater than among colored females. In a later part of this present section, we shall bring out in greater detail the age characteristics of this cancer mortality experience for the several color and sex classes, and with distinction of the several organs and parts. We quote below our general cancer death rates per one hundred thousand persons exposed in each of the color and sex classes for the various age periods. The accompanying chart shows the course of the cancer death rates in the experience according to age, for each of the color and sex classes.

In the introduction to this present section, we indicated that, in general, cancer mortality was lower among colored persons than among white persons, and that colored males showed relatively more favorable rates than colored females. Without any emphasis at this present time upon the organs or parts affected by cancer, we may now consider the general age characteristics of this disease.

A fairly significant rate is registered for the ages one to four years in the total experience. The cancer rate declines thereafter to its minimum at ten to fourteen years of age and then rises, at first gradually, but afterward in heavy increments up to the latest age period in this series. The same general characteristics of the age course of cancer mortality are observed for white males as for white females, with the exception that the

Death Rates per 100,000 Persons Exposed, Classified by Age Periods. White and Colored Groups Compared for Each Sex Class

Experience of Metropolitan Life Insurance Company, Industrial Department, 1911 to 1916



upward slope of the curve for white females is very much sharper for the ages beyond 25 years. The cancer death rates for colored persons under 25 years of age are, for the most part, very low, and fluctuate somewhat irregularly. Beginning with the age period 25 to 34 years, however, there is a constantly increasing rate, up to the highest age period recorded in this series.

TABLE 3

Mortality from Cancer, all forms, classified by color, by sex, and by age period. Deathrates per 100,000 persons exposed, 1911 to 1916. Experience of Metropolitan Life Insurance Company, Industrial Department, 1911 to 1916

Age Period.	Persons.	White.		Colored.	
		Males.	Females.	Males.	Females.
All ages—one and over ..	70.0	50.4	88.4	31.0	87.8
1 to 4	3.7	3.7	4.0	2.6	2.5
5 to 9	1.4	1.5	1.6	1.0	1.0
10 to 14	1.3	1.5	1.4	.3	.6
15 to 19	2.8	2.8	2.8	2.2	2.9
20 to 24	4.1	4.6	3.7	1.3	6.3
25 to 34	15.7	8.8	18.5	7.5	33.2
35 to 44	76.2	38.3	99.3	30.0	118.1
45 to 54	198.6	147.0	238.6	84.7	238.7
55 to 64	382.5	356.2	423.2	183.1	359.1
65 to 74	617.2	625.3	665.1	230.5	433.4
75 and over	818.2	822.8	863.9	384.1	580.7

COLOR RATIO OF CANCER MORTALITY

White males show emphatically higher cancer death rates at every age period than were recorded for colored males. Comparisons between the cancer death rates of white and colored females are practicable beginning with the age period 25 to 34 years. Between 25 and 44 years, the cancer death rate of white females was decidedly lower than the rate for colored females. Between 45 and 54 years, the rates were practically the same. Beginning with the age period 55 to 64 years and continuing to the highest age period in the table, we observe that the cancer death rates of white females were much higher than the rates for colored females. These differences in the total cancer death rates of white and colored females are to be accounted for, as will be shown later, by the higher mortality from cancer of the generative organs among colored females.

SEX RATIO OF CANCER MORTALITY

We have seen that among white lives the cancer death rate of females was practically one and two-thirds that of males. Cancer mortality of white males exceeds that of white females only for cancer of the buccal cavity, where the rates are 4.6 and .9 per 100,000 persons exposed, respectively; for cancer of the skin, where the rates are 2.2 and 1.6 respectively, and for the group of "cancers of other organs or of organs not specified." For cancer of the stomach and liver and of the peritoneum, intestines, and rectum, the death rates of white females were decidedly in excess of those for white males. In addition, white females showed a high death rate for cancer of the female genital organs (25.3 per one hundred thousand) and for cancer of the breast (11.7 per one hundred thousand). Practically the same general remarks apply to the comparative cancer death rates of colored males and colored females when compared with respect to the several organs affected by malignant growths.

There are no important differences in the cancer mortality of the two sexes among white lives under 25 years of age. Beginning with the age period 25 to 34 years, however, the cancer death rates of white females exceed those of white males substantially, up to and including the age period 55 to 64 years. Thus at the age period 35 to 44 years the rate for white males was only 38.6 per cent of that for white females. After age 65, the disproportion between the rates for the two sexes among white lives is not so great.

The excess of the cancer death rate of colored females over the rate for colored males is much greater than was observed, age period by age period, for white lives. Thus between 25 and 45, the rate for colored males was only about one-fourth as great as for colored females. In the following table, we give, first, a statement of the ratio of cancer mortality between the two races and, second, the sex ratio according to age period:

TABLE 4

Mortality from cancer (all forms). Percentages: Colored of White Mortality by Sex; Male of Female Mortality by Color; Classified by Age Period. 1911 to 1916. Experience of Metropolitan Life Insurance Company, Industrial Department, 1911 to 1916.

Age Period.	Per Cent. Colored of White Mortality.		Per Cent. Male of Female Mortality.	
	Males.	Females.	White.	Colored.
All Ages—One and Over	61.5	99.3	57.0	35.3
1 to 4	70.3	62.5	92.5	*
5 to 9	66.7	62.5	93.8	*
10 to 14	20.0	42.9	107.1	*
15 to 19	78.6	103.6	100.0	*
20 to 24	28.3	170.3	124.3	*
Ages 25 and Over	43.0	80.1	70.5	37.9
25 to 34	85.2	179.5	47.6	22.6
35 to 44	78.3	118.9	38.6	25.4
45 to 54	57.6	100.0	61.6	35.5
55 to 64	51.4	84.9	84.2	51.0
65 to 74	36.9	65.2	94.2	53.2
75 and over	46.7	67.2	95.2	66.1

*Insufficient data.

COMPARISON OF CANCER DEATH RATES AMONG INSURED WAGE EARNERS WITH RATES FOR POPULATION OF EXPANDING REGISTRATION AREA OF THE UNITED STATES

For both males and females, at all ages one and over combined, the cancer death rates of white lives in the insurance experience are substantially lower than the rates recorded in the Registration Area of the United States. This favorable ratio for the cancer mortality experience of insured wage earners does not hold for all of the age periods. Thus, the cancer death rate among white male wage earners is, in general, lower than the rate for males in the general population only at ages under 35 years. Among white insured females, the cancer death rate is lower than the rate among females in the general population only between 20 and 35 years of age.

The cancer death rate among both white males and white females of the insurance experience was higher than the rate for males and females in the Registration Areas at these age periods,

beyond 35 years of age, where cancer is of the most importance as a cause of death. It should be remarked also that male wage earners show much greater percentages of excess in cancer mortality than do females in wage earners' families. The following table sets forth these comparative facts of cancer mortality:

TABLE 5

Mortality from cancer (all forms). Death rates per 100,000 persons exposed. Classified by sex and by age period. Insured white lives in experience of Metropolitan Life Insurance Company, Industrial Department (1911 to 1916) and general population experience of expanding registration area of the United States (1910 to 1915).

Age Period.	Males.			Females.		
	M. L. I. Co. (White).	U. S. Reg. Area.	Per Cent. M. L. I. Co. of Reg. Area.	M. L. I. Co. (White).	U. S. Reg. Area.	Per Cent. M. L. I. Co. of Reg. Area.
All ages—one and over	50.4	62.2	81.0	88.4	97.9	90.3
1 to 4	3.7	3.6	102.8	4.0	3.1	129.0
5 to 9	1.5	2.0	75.0	1.6	1.4	114.3
10 to 14	1.5	1.7	88.2	1.4	1.4	100.0
15 to 19	2.8	2.9	96.6	2.8	2.6	107.7
20 to 24	4.6	4.4	104.5	3.7	4.6	80.4
25 to 34	8.8	9.3	94.6	18.5	20.8	88.9
35 to 44	38.3	31.9	120.1	99.3	89.0	111.6
45 to 54	147.0	109.8	133.9	238.6	227.0	105.1
55 to 64	356.2	280.3	127.1	423.2	406.9	104.0
65 to 74	625.3	503.4	124.2	665.1	607.0	109.6
75 and over	822.8	710.2	115.9	863.9	828.2	104.3

POSSIBLE RELATION OF CANCER TO ECONOMIC CONDITION OR SOCIAL STATUS

At this point in the discussion, brief reference may be made to the possible relation between the incidence of cancer mortality and economic status, as indicated in a paper recently published on this subject (2). The following table shows the main facts of an investigation based upon the comparative mortality experience of the Ordinary, Intermediate, and Industrial Departments of the Metropolitan Life Insurance Company during the three years 1914, 1915, and 1916. White lives only were included in this investigation. The Ordinary Department policyholders are drawn from higher economic strata of the population

than are the Intermediate group. The Industrial policyholders form the third class or group in order of material circumstance. In order to eliminate the slight effect of medical selection in the Ordinary and Intermediate groups with respect to cancer, we considered only the mortality in these classes on business in force at least five years.

TABLE 6

Cancer claim rates per hundred thousand mean in force. Ordinary and Intermediate departments, first five years of issue excluded, compared with Industrial Department, all years of issue combined. Composite mortality experience 1914, 1915, and 1916. White lives. By sex and by age period.

Sex and Age Period.	Ordinary Department.	Intermediate Branch.	Industrial Department.
MALES:			
Ages 25 and over	83.5	70.3	140.0
25 to 34	12.0	8.7	9.7
35 to 44	33.4	41.8	37.5
45 to 54	104.3	107.6	154.1
55 to 64	276.5	295.1	368.0
65 and over	662.5	645.3	679.2
FEMALES:			
Ages 25 and over	141.6	115.1	197.7
25 to 34	31.4	25.4	17.8
35 to 44	71.6	87.8	98.9
45 to 54	213.5	206.7	235.8
55 to 64	353.6	422.1	429.6
65 and over	313.1	1,009.8	707.5

In this table, *claim rates* per one hundred thousand mean in force for the several departments are compared. Actual experience demonstrates that very little error is involved in a comparison of mortality rates based upon the number of claims reported and the mean number of policies in-force if such data are related strictly to age periods. The comparison is valid therefore as above given. Because of the heavy representation of policyholders at the ages under 45 years, with a small number of deaths, leading to aberrant cancer death rates for these ages, the Intermediate rates for both males and females at all ages are apparently the lowest. At the ages beyond 45 years, where cancer mortality is numerically significant, the Industrial group showed the highest rate, the Ordinary the least, and the Intermediate a

rate between the other two. As a result of an extended consideration of the data developed in this inquiry into the possible relation of cancer and economic condition, it was concluded that:

1. The current medical opinion that there is a strong association between low economic status and a low cancer death rate is in all probability unfounded.

2. The cancer mortality rate at the ages where the cancer rate is significant decreases as we go up in the economic scale.

3. This is true for both sexes and by age period where sufficient data are available.

4. This conclusion is not conditioned by the effect of varying amounts of medical selection in the three groups considered.

TREND OF THE CANCER DEATH RATE

Medical literature of the past few years contains much controversy on the question whether mortality from cancer is actively increasing or not. One school of research holds "that the mortality from cancer is increasing at a more or less alarming rate throughout the entire civilized world and that this increase implies most serious consequences, present and future, to the populations concerned" (3). Another group of statisticians holds that "the reported mortality from cancer is increasing in almost every part of the world, but the real mortality, if increasing at all, is certainly not increasing with equal rapidity. . . . The cumulative evidence that improvements in diagnosis and changes in age composition explain away more than half, and perhaps all of the apparent increase in cancer mortality, rebuts the presumption raised by the figures and makes it probable, though far from certain, that cancer mortality is not increasing" (4).

It is not the purpose of this section to take sides in the controversy. It is desired to offer the mortality records of the present investigation only as a contribution to the available supply of trustworthy data on the trend of cancer mortality. The following table shows the rates per 100,000 persons exposed in each of the color and sex classes within the scope of this inquiry for the years 1911 to 1916:

TABLE 7

Mortality from cancer, all forms, classified by color and by sex. Death rates per 100,000 persons exposed. Single years in period 1911 to 1916. Experience of Metropolitan Life Insurance Company, Industrial Department.

Year.	Persons.	White.		Colored.	
		Males.	Females.	Males.	Females.
1911 to 1916	70.0	50.4	88.4	31.0	87.8
1916	70.3	51.8	87.2	36.5	86.1
1915	70.9	50.7	89.8	29.0	90.4
1914	69.8	50.7	87.9	28.0	88.3
1913	70.5	51.4	87.5	32.4	93.6
1912	70.3	47.8	91.7	30.7	86.3
1911	68.0	49.6	86.5	28.9	81.3

Cancer death rates in this present experience, covering six calendar years, and relating in all to fifty million years of life exposed to risk, show no decisive upward or downward tendency for all age classes combined. This is true for each color and sex group, but more decisively for the group of insured white females for whom the highest rates are recorded. The rates, by color and by sex, for the year 1911 are, to be sure, slightly lower than the figures for the entire six year period; this condition may be accidental and without significance. Considering all ages combined, therefore, there is no evidence presented in these figures from which an increasing mortality may be predicated with any certainty.

It would be more significant perhaps in this discussion to consider the trend of the cancer death rate during the six year period in a definite age period, especially one in which the cancer death rate is usually high. For this purpose we have chosen the age period 55 to 64 years. Table 8 shows for each one of the color and sex groups, the death rates from cancer (all forms) during the six year period.

This table shows very much the same trend in the age period 55 to 64 years as we found for all ages combined. The year 1911 was again a year of comparatively low cancer mortality. As the figures are compared for the individual years we find some variation with no clearly defined tendency toward increase or decrease. Our data, therefore, appear to confirm the idea

TABLE 8

Mortality from cancer (all forms) ages 55 to 64 years, classified by color and by sex. Death rates per 100,000 persons exposed. Single years in period 1911 to 1916. Experience of Metropolitan Life Insurance Company., Industrial Department.

Year.	Persons.	White.		Colored.	
		Males.	Females.	Males.	Females.
1916	386.4	358.0	427.4	218.3	339.9
1915	380.8	336.0	427.8	175.7	394.3
1914	390.9	385.0	423.3	167.7	351.7
1913	384.1	370.3	414.6	195.2	368.3
1912	381.9	334.1	443.2	176.4	325.4
1911	368.7	353.3	400.2	158.0	373.7

that the cancer death rate is not increasing, though if such a conclusion were drawn it could be but preliminary. There is need of reserve and caution in predicating any decisive opinion with regard to the real trend of cancer mortality during recent years, for a longer period of time will be required to collect authentic figures upon which a definite judgment can be based. Considerable analysis of cancer data according to age, sex, color, and organ or part affected will, in fact, be necessary before any final conclusions can be drawn as to the amount of increase, if any, in recent years. Another view of our data with respect to this question of cancer mortality increase is presented in the following table:

TABLE 9

Mortality from cancer (all forms). Percentage, death rate per 100,000 persons exposed in 1915–1916 of death rate in 1911–1912. Classified by color, sex and by significant age periods. Experience of Metropolitan Life Insurance Company, Industrial Department.

Age Period.	Persons.	White.		Colored.	
		Males.	Females.	Males.	Females.
Ages 25 and over	101.0	105.2	98.3	105.1	100.7
25 to 34	98.1	121.0	94.1	95.5	95.0
35 to 44	100.1	104.3	96.9	142.6	98.2
45 to 54	99.1	109.7	97.0	67.7	93.1
55 to 64	102.2	101.1	101.2	118.0	105.2
65 to 74	107.0	115.9	101.9	117.2	107.0
75 and over	101.0	104.1	104.4	110.7	48.6

We have eliminated in this table any superfluous references to ages under 25 years and in order to get at the heart of the matter have presented only the percentage which the cancer death rate in two years combined, 1915–1916, was of the death rate in two prior years, 1911–1912, at each age period, for each color and sex class.

Considering all persons in this mortality experience at ages 25 and over, there was an increase of only 1.0 per cent in the cancer death rate between the two periods compared. This figure is a composite of a variously weighted increase of 5.2 per cent for white males, a decrease of 1.7 per cent for white females, an increase of 5.1 per cent for colored males and a practically stationary rate for colored females. Considered according to age period, this increase of 1.0 per cent in the cancer death rate of all persons in this experience, aged 25 years and over, was a composite of a decrease of 1.9 per cent between 25 and 34 years, contributed very largely out of the experience of white and colored females, a practically stationary death rate between 35 and 44 years, which is, in itself, a composite of an increase for white and colored males and a decrease for white and colored females, and a slight decrease between 45 and 54 years. At this latter age period, we observe an increase of nearly ten per cent in the white male rate, a decrease of 3.0 per cent for white females, of 6.9 for colored females, and of 32.3 per cent for colored males. The major influence, however, in slightly lowering the cancer death rate of all persons between 45 and 54 years was, of course, the experience of the group of white females. Between 55 and 64 years, all classes in the mortality experience show an increase in the rate, highest for colored males and least for white males. The age period 65 to 74 years shows an increase of 7.0 per cent which is contributed very largely by the experience on male lives of both color groups. It should be remarked that the cancer experience of colored persons exerts but slight influence upon the ratio of increase of cancer mortality in the entire experience. In fact, for some of the age periods, the data on the increase of cancer mortality among colored persons are aberrant.

It will be seen from the foregoing table that considerable analysis of cancer facts according to age, sex, color, and by organ or part affected is necessary before any final conclusions are drawn as to the amount of real increase in cancer mortality, if any, in recent years. A discussion, in some detail, of the cancer mortality experience according to the organs or parts follows.

COLOR, SEX, AND AGE STATISTICS OF CANCER ACCORDING TO ORGANS OR PARTS

Cancer[2] of the stomach and liver

The deaths classified under this heading constituted, as was shown above, the most important subordinate group of specific types of cancer. Cancers of the stomach and liver were recorded in 37.6 per cent of all cancers in this entire experience. It should be remembered that this heading also includes cancers and other malignant tumors of the pharynx, the esophagus, and the gall-bladder.[3] The combined total of malignant growths of the pharynx and esophagus, however, numbers less than 5 per cent of the total in general practice, and does not, therefore, seriously affect the data for this title heading. In gall-bladder cancers the liver is frequently involved.

During the period of this present investigation, there were recorded 14,153 deaths from malignant growths of the stomach and liver. The death rate was 26.3 per 100,000 persons exposed. As shown in the following table, the facts vary considerably according to color, sex, and age period.

There is a higher death rate for this cause, without important exception, for white lives than for colored lives. Below 35 years, mortality from cancer of the stomach and liver is not numerically important. The death rate increases from a figure of 18.6 in the age period 35 to 44 years to the maximum in the highest age period in this series, 75 years and over. From 45 years of

[2] Cancer and other malignant tumors.

[3] The International list heading "Cancer of the Stomach, Liver," is somewhat misleading, inasmuch as cancers of other organs of the digestive system are classified under it.

age and onward, however, the death rate of white males for this disease is appreciably higher than the rate for white females, with the exception of the very highest age period, 75 years and over. Among colored persons the death rate from cancer of the stomach and liver is higher for males than for females between 55 and 74 years only. At the ages under 55 years, colored females show higher death rates from this cause than do colored males.

TABLE 10

Mortality from cancer of the stomach and liver, classified by color, by sex, and by age period. Death rates per 100,000 persons exposed, 1911 to 1916. Experience of Metropolitan Life Insurance Company, Industrial Department.

Age Period.	Persons.	White.		Colored.	
		Males.	Females.	Males.	Females.
All ages—one and over...	26.3	24.8	29.8	16.2	18.5
1 to 24...........	.3	.3	.3	.2	.6
25 to 34...........	3.0	2.9	2.7	3.3	5.0
35 to 44...........	18.6	18.1	20.1	13.8	16.5
45 to 54...........	67.2	77.0	67.7	44.0	44.8
55 to 64...........	168.5	193.3	168.4	108.0	99.5
65 to 74...........	276.0	303.6	288.7	125.3	122.1
75 and over........	334.7	339.1	353.3	164.6	217.8

COMPARISON OF DATA FOR CANCER OF THE STOMACH AND LIVER AMONG INSURED WAGE EARNERS AND AMONG THE POPULATION OF THE EXPANDING REGISTRATION AREA OF THE UNITED STATES

For both males and females at the ages where death rates from this cause are at all significant, a higher death rate is recorded among the group of insured wage earners than is observed in the Registration Area of the United States. Beginning with the age period 35 to 44 years, there is an excess of over 19 per cent, between 45 and 54 years, an excess of 32 per cent, and between 55 and 64 years, an excess of 29 per cent over the rates for males prevailing in the expanding Registration Area of the United States. The excess in the death rate of cancer of the stomach among insured females is not so great as in the case of insured males. In the following table, a comparison of the foregoing facts is given:

TABLE 11

Mortality from cancer of the stomach and liver. Death rates per 100,000 persons exposed. Insured white lives in experience of Metropolitan Life Insurance Company, Industrial Department (1911 to 1916) and General Population Experience of expanding registration area of the United States (1910 to 1915). Classified by sex and by age period.

Age Period.	Males.			Females.		
	M. L. I. Co. (White).	U. S. Reg. Area.	Per Cent. M. L. I. Co. of Reg. Area.	M. L. I. Co. (White).	U. S. Reg. Area.	Per Cent. M. L. I. Co. of Reg. Area.
All ages—one and over	24.8	30.5	81.3	29.8	32.1	92.8
Under 25	.3	.4	75.0	.3	.3	100.0
25 and over	65.8	56.4	116.7	63.0	61.4	102.6
25 to 34	2.9	3.0	96.7	2.7	3.9	69.2
35 to 44	18.1	15.2	119.1	20.1	18.2	110.4
45 to 54	77.0	58.4	131.8	67.7	62.1	109.0
55 to 64	193.3	150.2	128.7	168.4	147.5	114.2
65 to 74	303.6	254.3	119.4	288.7	253.6	113.8
75 and over	339.1	294.1	115.3	353.3	313.7	112.6

TREND OF THE DEATH RATE FROM CANCER OF THE STOMACH AND LIVER

Not much stress can be put on the figures showing the trend of the death rate in the period between 1911 and 1916. In the first place, the figures vary considerably from year to year, sometimes increasing, sometimes decreasing; also, cancer of the stomach and liver affects organs which are practically inaccessible for purposes of precise diagnosis. There must, therefore, be considerable uncertainty in the reliability of the diagnosis. It will be necessary, therefore, to wait for an extension in the period of observation before any definite tendency of the death rate from this form of cancer can be predicated. In the meanwhile as diagnostic facilities become more generally available and as the practice of making autopsies becomes more widespread it may be expected that the recorded death rates for cancer of the stomach and liver will show slight increases.

Cancer[4] of the female genital organs

Cancer of the female genital organs accounted for 28.6 per cent of all cancer deaths among white females. The very largest proportion of these cancers affected the uterus, with the ovaries and Fallopian tubes next in numerical importance.

In all, 7882 deaths from cancer of the female genital organs were recorded in the six year period of this study. The rate has significance only when the details are related to the number of females exposed. The 6499 cancers registered among white females corresponded to a rate of 25.3 per 100,000 such females

TABLE 12

Mortality from cancer of the female genital organs, classified by color and by age period. Death rates per 100,000 persons exposed, 1911 to 1916. Experience of Metropolitan Life Insurance Company, Industrial Department.

Age Period.	White Females.	Colored Females.
All ages—one and over	25.3	37.9
1 to 19	.2	.2
20 to 24	1.0	1.3
25 to 34	7.9	17.7
35 to 44	43.4	62.9
45 to 54	85.7	109.7
55 to 64	109.4	131.6
65 to 74	110.3	131.5
75 and over	93.8	145.2

exposed and the 1383 deaths among colored females to a rate of 37.9 per 100,000 exposed. Under the age of 25 years, there was no significant mortality from this cause. Beginning with the age period 25 to 34 years, however, there was a quite considerable rate of mortality, 7.9 per 100,000 for white females and 17.7 for colored females. This excess in the mortality rate from cancer of the female genital organs among colored females is decidedly marked at all of the age periods in this series. The maximum rates of mortality from this cause appear at the older ages. The preceding table gives the facts according to age classes among white and colored females.

[4] Cancer and other malignant tumors.

COMPARISON OF DEATH RATES FROM CANCER OF THE FEMALE GENITAL ORGANS IN INSURANCE EXPERIENCE ON WHITE LIVES AND IN EXPERIENCE OF THE POPULATION OF THE EXPANDING REGISTRATION AREA

Again, it is found that white females in wage earners' families show a decided excess in the mortality rate from cancer of the female genital organs over the rates recorded for females in the general population of the expanding Registration Area. The following table gives a comparative view of these death rates:

TABLE 13

Mortality from cancer of the female genital organs. Death rates per 100,000 persons exposed. Insured white females in experience of Metropolitan Life Insurance Company, Industrial Department (1911 to 1916) and females in general population experience of expanding registration area of the United States (1910 to 1915). Classified by age period.

Age Period.	Females.		
	M. L. I. Co. (White).	U. S. Reg. Area.	Per Cent. M. L. I. Co. of Registration Area.
All ages—one and over	25.3	25.0	101.2
Under 25	.4	.5	80.0
25 and over	53.2	47.7	111.5
25 to 34	7.9	8.4	94.0
35 to 44	43.4	34.7	125.1
45 to 54	85.7	75.6	113.4
55 to 64	109.4	99.9	109.5
65 to 74	110.3	103.2	106.9
75 and over	93.8	98.3	95.4

The greatest excess in mortality from cancer of the female genital organs among white females in the families of insured wage earners was recorded between 35 and 44 years. The percentage of excess in mortality from this cause among white females decreases with advancing age, and at the very late ages in life the rates for both the insurance and population experience tend to approximate each other.

TREND OF THE DEATH RATE FROM CANCER OF THE FEMALE GENITAL ORGANS

The figures available show neither a favorable nor unfavorable tendency of the death rate from cancer of the female genital organs. There are slight variations from year to year.

Cancer[5] *of the breast*

In view of this very small number of male deaths from cancer of the breast, we shall concentrate our attention entirely upon

TABLE 14

Mortality from cancer of the breast. Number and percentage of total deaths from cancer of the breast in each color and sex class. Experience of Metropolitan Life Insurance Company, Industrial Department, 1911 to 1916

Color and Sex.	Number of Deaths.	Per Cent.
All classes	3,579	100.0
White males	31	.9
White females	3,004	83.9
Colored males	9	.3
Colored females	535	14.9

the statistics for white and colored females. The following table gives the death rates for white females and colored females at the several age periods.

TABLE 15

Mortality from cancer of the breast, females classified by color and by age period. Death rates per 100,000 persons exposed, 1911 to 1916. Experience of Metropolitan Life Insurance Company, Industrial Department

Age Period.	White Females.	Colored Females.
All ages—one and over	11.7	14.7
1 to 24	†	.1
25 to 34	2.5	3.3
35 to 44	17.4	18.9
45 to 54	36.9	41.9
55 to 64	47.6	59.9
65 to 74	76.0	96.3
75 and over	108.8	118.0

† Less than 0.05 per 100,000.

[5] Cancer and other malignant tumors.

There is a constantly rising death rate with age from this cause for both white and colored females. There is also a decided excess in the death rate among colored over white females although this excess among colored females is not so marked as it was for cancer of the female genital organs.

Throughout the six years under examination, there was, with the exception of the year 1915 for colored women, a practically stationary death rate for cancer of the breast. In the following table, we quote our experience for each of the years from 1911 to 1916:

TABLE 16

Mortality from cancer of the breast, females classified by color. Death rates per 100,000 persons exposed. Single years in period 1911 to 1916. Experience of Metropolitan Life Insurance Company, Industrial Department

Years.	White Females.	Colored Females.
1911 to 1916	11.7	14.7
1916	11.8	14.9
1915	12.5	17.4
1914	11.4	12.3
1913	11.9	14.3
1912	12.1	15.6
1911	10.2	13.5

Cancer of the breast seems not to have as heavy a mortality among white insured females as it does among females in the population of the expanding Registration Area of the United States. Between 35 and 44 years, there is practically the same death rate from this cause in both experiences. But between 55 and 64 years the group of insured females in wage earners' families shows much lower death rates from this cause than were found for females in the Registration Area of the United States. It should be recalled at this point that the data for the expanding Registration Area comprise a small proportion of colored women. This fact, considering the higher death rate from cancer of the breast among negro women than among white women, may account in some measure for the higher mortality from this cause in the population experience over the exclusively white insurance experience. The following table affords a comparative view of the statistics for cancer of the breast among insured

white females and among females in the general population of the expanding Registration Area:

TABLE 17

Mortality from cancer of the breast. Death rates per 100,000 persons exposed. Classified by sex and by age period. Insured white females in experience of Metropolitan Life Insurance Company, Industrial Department, 1911 to 1916, and females in general population experience of expanding registration area of the United States, 1910 to 1915

Age Period.	M. L. I. Co. (White).	U. S. Reg. Area.	Per Cent. M. L. I. Co. of Registration Area.
All ages—one and over	11.7	15.5	75.5
Under 25	†	.1	—
25 and over	24.7	29.7	83.2
25 to 34	2.5	2.7	92.6
35 to 44	17.4	17.5	99.4
45 to 54	36.9	41.3	89.3
55 to 64	47.6	61.0	78.0
65 to 74	76.0	81.0	93.8
75 and over	108.8	130.6	83.3

†Less than 0.05 per 100,000.

Cancer[6] of the peritoneum, intestines, and rectum

Cancer of the intestines constituted the very largest number of the 4482 deaths under this head. The death rates by age, sex, and color for this cause are shown below:

TABLE 18

Mortality from cancer of the peritoneum, intestines, and rectum, classified by color, by sex, and by age period. Death rates per 100,000 persons exposed, 1911 to 1916. Experience of Metropolitan Life Insurance Company, Industrial Department

Age Period.	Persons.	White.		Colored.	
		Males.	Females.	Males.	Females.
All ages—one and over	8.3	6.6	10.5	3.4	7.2
1 to 24	.4	.4	.3	.2	3
25 to 34	2 2	1.6	2.2	1.8	3.9
35 to 44	7.8	6.0	8.8	4.0	11.7
45 to 54	22.1	19.7	25.8	7.5	19.9
55 to 64	43.9	43.6	50.5	13.2	21.1
65 to 74	83.1	80.8	95.0	28.4	31.7
75 and over	109.5	86.5	130.6	96.0	36.3

[6] Cancer and other malignant tumors.

Mortality from this form of cancer also increases with advancing age. White males show lower death rates at all significant ages than do white females. The colored male death rate from this cause is also decidedly lower than the rate for colored females. It would be well to recall at this present time that in this investigation tuberculous disease of the abdominal organs also shows a higher death rate among females of both white and colored races, especially at the ages of the childbearing period, 15 to 45 years. This fact suggests the possible influence of puerperal traumata as contributing causes in exciting both

TABLE 19

Mortality from cancer of the peritoneum, intestines, and rectum. Death rates per 100,000 persons exposed. Classified by sex and by age period. Insured white lives in experience of Metropolitan Life Insurance Company, Industrial Department, 1911 to 1916, and general population experience of expanding registration area of the United States, 1910 to 1915

Age Period.	Males.			Females.		
	M. L. I. Co. (White).	U. S. Reg. Area.	Per Cent. M. L. I. Co. of Reg. Area.	M. L. I. Co. (White).	U. S. Reg. Area.	Per Cent. M. L. I. Co. of Reg. Area.
All ages—one and over	6.6	8.6	76.7	10.5	10.4	101.0
Under 25.........	.4	.5	80.0	.3	.3	100.0
25 and over.......	16.9	15.5	109.0	21.9	23.5	93.2
25 to 34..........	1.6	2.1	76.2	2.2	2.6	84.6
35 to 44..........	6.0	5.5	109.1	8.8	9.1	96.7
45 to 54..........	19.7	15.2	129.6	25.8	24.6	104.9
55 to 64..........	43.6	37.6	116.0	50.5	51.8	97.5
65 to 74..........	80.8	67.6	119.5	95.0	89.5	106.1
75 and over.......	86.5	80.9	106.9	130.6	117.6	111.1

tuberculous and cancerous processes in the main adult ages among females.

At the ages in which the death rate from cancer of the peritoneum, intestines, and rectum is considerable, insured white males show higher rates than do males in the general population. Between 45 and 75 years of age, the excesses in the rates range from 16 to 30 per cent. Insured white females, on the other hand, show no very marked tendency to depart from the general population experience. At some age periods, the death rate among insured white females is slightly more favorable and at

other age periods slightly less favorable than among females in the general population. The above table gives a comparative view of the data for males and females of the insurance and population experience, considered according to age period.

The data indicate a practically stationary death rate from this cause between 1911 and 1916.

Other forms of cancer[7]

Discussion in great detail of cancers of organs and parts of the body, in addition to those covered by the preceding text, is not justified for many reasons. There are, however, many interesting and important age, sex, and color relations disclosed in the study of "other forms of cancer" which should not be passed without brief comment. These we shall discuss briefly in relation to cancers of the buccal cavity, of the skin, and of all "other organs or of organs not specified" in the order named.

Cancer[8] *of the buccal cavity*

Cancer of the buccal cavity, including cancer of the maxillae, caused 1353 deaths during the six year period 1911 to 1916. This corresponds to a death rate of 2.5 per 100,000 exposed. Of the 1353 deaths, 1229 were those of white policyholders and 124 of colored persons. The death rate per 100,000 exposed was 2.6 for white lives as compared with 1.9 for colored lives. Cancer of the buccal cavity shows a very strong sex incidence. Of the 1353 deaths, 1064 were those of males and only 289 those of females. This excess among males applies to both white and colored lives; especially to the former, among whom male mortality (4.6) was more than five times that of females (0.9). White males show a death rate almost twice that for colored males (4.6 as compared with 2.5) and in the female experience also the rate for colored women (1.3) exceeds that for insured white women (0.9). This comparison for colored policyholders, how-

[7] Cancer and other malignant tumors.

[8] Cancer and other malignant tumors.

ever, is hardly valid on account of the small number of deaths involved.

The great majority of the deaths classified as due to cancer of the buccal cavity were reported under the terms "cancer of the jaw," "cancer of the tongue," "cancer of the lip," and "cancer of the mouth" without more definite designation.

There is no pronounced upward or downward trend shown for cancer of the buccal cavity in the industrial experience of this Company during the six year period covered by this report. The death rate was 2.3 per 100,000 exposed in the first year of the sexennium as compared with 2.4 for the last year. The rates in the general population are slightly higher than those for the insured. This is accounted for largely by differences in the age distribution of the two populations. As a general proposition only 5 per cent of the deaths from this disease occur among persons under 50 years of age. The great bulk of the deaths are those of men between the ages of 50 and 79.

Cancer[8] *of the skin*

Skin cancers were reported as causes of death in 938 cases in the mortality experience of insured wage earners covering the six year period 1911 to 1916, corresponding to a death rate of 1.7 per 100,000 exposed. Little fluctuation is shown in the rate when the years of the period are compared with one another. Cancer of the skin is, almost altogether, confined to white persons. In the Metropolitan experience 887 of the 938 deaths were those of white persons, the total white death rate being 1.9 per 100,000 exposed as compared with 0.8 for the colored insured. In the matter of sex incidence a considerable excess in the white experience is shown for males over females; the rate for the former being 2.2 as compared with 1.6 for the latter. The rates for colored policyholders are not so significant when compared by sex on account of the small number of deaths involved.

As with cancers of the buccal cavity, only a comparatively small percentum of the deaths from skin cancers are those of

persons under 50 years of age. The mortality is bulked between the ages of 60 and 79 years. When we compare buccal and skin cancers by age groups we find that there is a much higher mortality, relatively, from the skin cancers in extreme old age than from cancers of the buccal cavity.

A lower death rate at all ages for this form of cancer is found among the insured than among the general population. The same explanation for this obtains as for cancer of the buccal cavity, namely, the more favorable age distribution of policyholders. Like that of the insured group, the general population experience for this cause of death shows little change in the rate during the sexennium to which this report relates. The great majority of the deaths charged to this title heading were reported in one of the following ways: cancer of the face, cancer of the nose, cancer of the skin, rodent ulcer, and epithelioma (location not indicated).

CANCER AND OTHER MALIGNANT TUMORS OF OTHER ORGANS OR OF ORGANS NOT SPECIFIED

The above is a *residual* heading under which are classified all deaths from cancers that cannot be definitely assigned to one of the preceding titles; it also includes cancers in which no statement is given of the location or original seat of the disease. The more definite titles included are the cancers of the bladder, of the prostate, of the pancreas, of the kidneys and suprarenals, of the lung and pleura, of the bones (jaw excepted), of the larynx, of the brain, of the testes, of the parotid gland, and of the spleen.

This title constitutes an important cause of death numerically in the present experience. No fewer than 5,279 deaths were charged to it and the corresponding rate was 9.8 per 100,000 exposed. With the exception of cancer of the stomach and liver, and cancer of the female genital organs, more deaths are charged to this residual title than to any of the other separate headings relating to malignant growths.

There was only a very small fluctuation in the death rate during the several years which constitute the period to which

this report relates. This same phenomenon is in evidence for the expanding Registration Area of the United States. The death rate for this cause, however, is considerably higher in the latter experience for these forms of cancer than among the insured group.

The total death rate for the insured white experience was 10.1 per 100,000 exposed as compared with 7.6 for the colored experience. In each, the mortality among males was higher than among females, although this is much more pronounced among the white policyholders than among the colored. This excess for males is accounted for, in part, by the fact that all deaths from cancers of the male genitals are classified under this heading.

REFERENCES

(1) Mortality from Cancer, etc., Washington, 1914. Published by Department of Commerce, Bureau of the Census, Sam. L. Rogers, Director.

(2) Knight, Augustus S. and Dublin, Louis I.: The Relation of Cancer to Economic Condition. Read before the Association of Life Insurance Medical Directors of America, Worcester, Mass., October 17, 1917. Reprinted by Metropolitan Life Insurance Company, New York, 1917.

(3) Hoffman, Frederick L.: The Mortality from Cancer Throughout the World, Newark, New Jersey, 1915, p. 218; The Mortality from Cancer in the Western Hemisphere, Jour. Cancer Research, 1916, i, 21.

(4) Willcox, Walter F.: On the Alleged Increase of Cancer. Quarterly Publications of the American Statistical Association, September, 1917, p. 756; Jour. Cancer Research, 1917, ii, 267.

THE LIPOIDS IN TUMORS OF THE DENTAL REGION

KAETHE W. DEWEY

From the Research Laboratory of the College of Dentistry, University of Illinois

Received for publication, November 4, 1918

In view of the enormous amount of investigative work which has been done on the lipoids, and in particular on cholesterol, the contributions to the study of their occurrence in tumors are surprisingly scanty. With the hitherto customary practice of considering most pathological conditions of the teeth and the peridental tissues as somewhat outside the pale of general pathology, tumors of the dental region have received little, if any, attention in the study of this interesting question.

The tumors richest in cholesterol next to cholesteatomas are the hypernephromas; also adenomas of the kidney may contain large amounts of it; cholesterolesters are the chief substance infiltrating xanthoma cells and pseudoxanthoma cells. The pigments to which these tumors owe in part their characteristic color, also the pigments found in chloromas, are considered by some authorities to belong to the lipochromes, that is, to be of a lipoid nature.

As elsewhere in pathological and physiological infiltration, cholesterol occurs in tumors as esters and more or less loose combinations with fatty acids. White (1), in examining carcinomas and sarcomas for their content in crystalline substances, found five types of crystals, four of which were cholesterol, either pure or combined, and which could be differentiated by characteristics of the crystalline form and variations in the melting point. An interesting observation to which White has called attention is that gallstones, which consist largely of cholesterol, are two and a half times as common in patients suffering from carcinoma as in patients of the same age suffering from other diseases, and this frequency is independent of the site of the primary

growth. Ciaccio (2) states that cholesterol, or "lecithin" as he calls it, is constantly found in tumor cells and that it is directly related to the malignancy of the tumor, so that it is especially abundant in carcinomas and sarcomas. Podwyssotzky (3) reported the finding of cholesterol crystals in an ulcerated cancroid of the maxilla and suggested that they may be a stage of metamorphic alteration, namely hyaline degeneration passing over into the crystalline state.

The lipoid substances discussed in the literature are, as a rule, simply and briefly spoken of as "fat." Although it is now generally admitted that by far the largest part of this so-called "fat" consists of cholesterol in the form of esters or of more or less loose combinations with fatty acids and other lipoids, the infiltration of tissues with these lipoids is still almost universally called "fat" infiltration. For greater precision in terminology, Aschoff (4) proposed the names of "cholesterolester" infiltration and "glycerinester" infiltration as the main types and, in fact, the only ones which come under consideration. Kaiserling (5) adopted a classification of liposis, lipoidosis, and lipo-lipoidosis, according to the substance that is in preponderance. However, this does not clear up the confusion, by any means. Very frequently, cholesterolesters and glycerinesters occur simultaneously in the same tissue and often in the same cell. There is not yet even any unanimity in the use of the terms "lipoid" and "fat." These are applied very loosely by some authorities, while others make sharp distinctions and refer to neutral fat as genuine or ordinary fat. Then there are some who insist on dividing the fatlike substances not into two, but into three classes. That the physicochemical properties, however, are sufficiently marked and that the group distinctions may be made from their reactions to staining methods is best illustrated in tables which Aschoff (4) and Kawamura (6) have published.

Until quite recently the chief stains for lipoids were osmic acid and Sudan III. The latter is an excellent stain and the most satisfactory means to demonstrate all fats or all lipoids, but a differentiation beyond that of the red staining neutral fat

from all the lipoids staining yellowish red cannot be made. As to osmic acid, it is reduced only by oleic acid, olein, and lecithin. Now, however, we possess several new staining methods by which certain lipoids in tissues may be fixed and others eliminated. By employing all these staining methods, and by making use of the polarizing microscope, we are enabled to establish the nature of the different lipoids in tissues with a fair degree of accuracy and in a much larger measure than was hitherto possible. This is the conclusion arrived at in studying tumors and cysts of the dental region for their lipoid content.

The specimens were obtained from Dr. Moorehead's clinic for oral surgery, of the University of Illinois, and included endotheliomas, fibromas, giant-cell sarcomas, cysts and ordinary hypertrophy of the gum tissue.

TECHNIC

Concerning the technic I may briefly state that frozen sections were made from the tissues, fixed in 10 per cent formalin, and stained with Sudan III, Nile blue sulphate, and neutral red. Other pieces and sections were prepared by the methods devised by Ciaccio (7), Smith (8), Dietrich (9), and Fischler (10). These are indispensable in any attempt to differentiate lipoids in tissues. By Fischler's method, fatty acids and soaps are demonstrated. The Smith-Dietrich and Ciaccio methods are based on the observation that in tissues treated with potassium chromate only some of the lipoid substances are chromated, namely the more or less loose combinations of cholesterol with fatty acids and other lipoids. Thus fixed, they are insoluble in fat solvents used for paraffin embedding (Ciaccio), or lake hematoxylin as in Weigert's method of staining the myelin sheath (Smith-Dietrich). The glycerinesters or neutral fat, as well as the stable cholesterolesters, are not fixed by chromium salts. The polarizing microscope reveals the physical properties as follows: cholesterolesters and mixtures of cholesterol with fatty acids and certain lipoids occur in the form of doubly refractive droplets. The glycerinesters, and their mixtures with choles-

terol, fatty acids, and soaps are not doubly refractive. The double refraction disappears on heating and reappears or does not reappear with some lipoids. The melting point varies according to the mixture. Aschoff's and Kawamura's tables are invaluable guides in histomorphologic studies of the lipoids.

STUDY OF SPECIMENS

The largest amount of such substances was found in an angio-endothelioma of the submaxillary gland. The tumor, which was the size of a walnut, was surrounded by a thick fibrous capsule. Cut surfaces showed great mottling, as there were large irregular blackish areas, alternating with gray, spongy-looking tissue and with regions of dense white tissue.

In unstained frozen sections there are many large and small blood-spaces and blood effusions, some of the spaces being empty. Everywhere in these areas there are scattered singly, or in small clumps, yellow, yellowish red, brown, or black erythrocytes and leukocytes. The red cells are often quite homogeneous. The leukocytes are coarsely granular and sometimes disintegrated. The pigmented cell collections are near the margin of the spaces. All spaces have well defined walls. Besides the pigmented cells, there is blackish brown pigment about most of the vessels, often forming a thick coat. Under the low power lens, it looks like iron filings; under the high power lens it is found to be a granular material. These pigmented granules pervade almost the entire tumor tissue and form fine lines about the cells. There is no pigment about the capsule.

In sections stained with Sudan III, there is an amazing amount of orange red material. The tumor cells look as if embedded in a solid trabecular frame-work of lipoid substances. Also the individual cells in large portions of the tumor are encircled by a finely granular yellow material. All the large and small spaces are surrounded by thick coats of the same material in all shades from light yellow to dark orange red. Sudan III corresponds to the fine black material resembling iron filings in unstained sections, but is far in excess of this pigment. A small portion remains

black. The broad connective tissue capsule contains large amounts of a reddish-stained material. The plasma in the blood-spaces is orange red and contains distinct droplets. The structure of the orange red trabeculum is not distinguishable. A conspicuous feature is the large, round, morula-like masses of bright orange red globules lying in spaces, some of which are filled with orange red coagulated plasma. The droplets are small and uneven, and entirely unlike neutral fat droplets. These round morula-like masses are apparently greatly enlarged leukocytes completely transformed into lipoid material. There is no nucleus visible, and although they are sharply outlined from the homogeneous protoplasm which has sometimes shrunk away from them, no cell membrane is discernible. Sometimes the lipoid globules are dispersed from their original boundary. Very large lipoid transformed cells are also found in rows about some vessels, and single red granular cells are scattered in regions of blood effusions. In view of this enormous amount of lipoids throughout the tumor, including the fibrous capsule, it is striking that the tumor cells themselves are free from stainable lipoids.

Nile blue stained sections reveal the same enormous amount of lipoids as sections stained with Sudan III. There is no bright red stained material denoting neutral fat. The trabeculum appears in a lavender color with a reddish tinge. The homogeneous lipoid erythrocytes, generally enlarged, are a dirty brownish red, and the huge morula-like leukocytes are lavender. The clumps of yellow granular leukocytes or homogeneous erythrocytes seen in unstained sections remain so in Nile blue stained sections. They are sometimes intermingled with reddish granular droplets. The vessels are surrounded by a purplish, finely granular material. The endothelial cells lining the spaces are filled with fine purplish-red staining granules. They are sometimes slender, sometimes greatly enlarged, and occasionally disintegrated.

There are within the outer zone of the capsule solid, round, or ringlet shaped masses in large numbers which are newly formed blood-vessels in early stages of calcification. They are stained intensely blue or almost black. This deep blue coloring,

indicating fatty acids and soaps, is particularly interesting, because the vessel walls within the tumor tissue proper are not blue, but have shades varying from pinkish lavender to purple.

VARIOUS METHODS AND THEIR RESULTS.

Contradictory views have been expressed concerning the value of Nile blue. Some consider it as unreliable and unsatisfactory, others praise it as the most efficient of staining methods for lipoids. My experience with the stain has been that occasionally it is not practicable; namely, when the lipoids are present in extremely fine droplets and dispersed diffusely within a tissue. But wherever lipoids are present in appreciable amounts and are of different types, Nile blue is superior to Sudan III as a differentiating stain. In tissues very rich in lipoids, as is this endothelioma, one may obtain, almost at a glance, a fairly accurate estimate of the types of lipoids from the scale of shades, from red to deep blue. The coloring of some lipoids seems to change within twenty-four hours from the time of staining, at least in some cases. I have observed that particularly fatty acids and soaps may be decidedly reddish when freshly stained, and may next day appear in the characteristic dark blue tint. Also, the cholesterol mixtures are sometimes more reddish at first and gradually assume the purplish hue. After this initial change, the coloration is stable.

In sections prepared by Smith's method, only a part of the lipoids revealed by Sudan III and Nile blue are demonstrated. They are contained within large cells grouped about vessels and sometimes also in collections within vessels. These cells, leukocytes and endothelial cells, are bluish gray, the typical bright blue coloration not being observed in these sections. The yellow and black pigmented elements and structures are not changed. There are areas within the capsule in which streaks of a faintly bluish gray granular material are present. It is an interesting observation that if sections mordanted by Smith's method are stained with Sudan III, much more of the black material with the appearance of filings reappears in red, than is the case in un-

mordanted formalin-fixed sections. According to Kawamura's table, the lipoids in these sections which are positive with Smith's method may be considered as mixtures of cholesterol with fatty acids. They are anisotropic under the polarizing microscope. Double refraction is lost in heating the sections. These same substances are negative with Fischler's and Ciaccio's methods.

In sections prepared by the latter method, the only lipoids demonstrated are found almost exclusively within the broad connective tissue capsule. They appear as numerous homogeneous circular or crescent-shaped red stained masses which represent the degenerated thickened walls of capillaries. The same structures stain black with Smith's method and dark blue with Nile blue, and are positive with Fischler's staining method; hence they may be considered fatty acids and soaps. In the tissue of the tumor proper, there is very little material about vessels or elsewhere that is positive with Ciaccio's method.

Examination with the polarizing microscope shows that the lipoids occurring as doubly refractive crystals are limited to large cells described in the foregoing (fig. 1) which stain grayish blue with Smith's method, and lie in wreaths around the vessels, or in collections within them. These cells are completely filled with brilliant crystals. Some of them show the central cross before heating. Besides the large cells filled with crystals, there are smaller collections and single globules lying outside the cells. All these crystals disappear on slight heating and reappear on cooling. None of the different kinds of pigments appears as doubly refractive crystals. The lipoids occurring in streaks and masses in the capsule and in the trabeculum of the tumor proper have a brilliant silvery luster. According to Kawamura's table, these doubly refractive crystals, disappearing on heating, are probably mixtures of cholesterol with fatty acids; for cholesterolesters, having the same properties, may be ruled out because they are negative by Ciaccio's, Smith's, and Fischler's staining methods.

The findings in this tumor, therefore, illustrate the fact that all the methods combined for demonstrating the lipoids are adequate means to differentiate most of the lipoids that may be

present side by side in the same tissue. Smith's and Fischler's methods are apparently exceedingly reliable in demonstrating a group of lipoids classified as fatty acids and soaps.

A frequent observation in these tumors of the dental region is the presence of large amounts of lipoids in leukocytes. Apparently complete lipoid transformation of the cells takes place

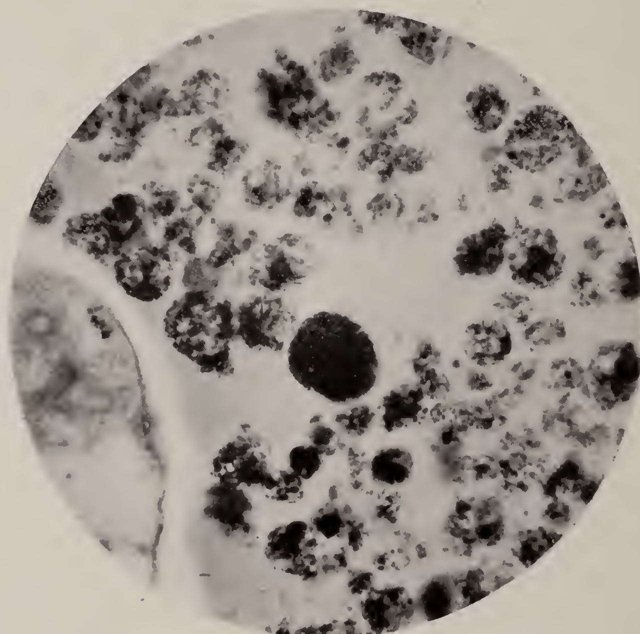


Fig. 1. Frozen Section Stained with Nile Blue

From an angioendothelioma. Huge leukocytes in a large space containing coagulated serum; they are completely filled with doubly refractive lipoid droplets (mixtures of cholesterol with fatty acids). × 280.

in this condition. In the endothelioma described in the foregoing, they are found in large and small collections within blood-spaces, in hemorrhagic areas, in layers encircling blood-vessels, in short, wherever slowing of the blood-stream or stasis has caused a gathering of white cells. The cells filled with lipoids are sometimes enormously increased in size. These and the endothelial cells lining blood-vessels are the only cells in this

tumor which morphologically reveal lipoid substances. The tumor cells themselves are free from demonstrable anisotropic droplets under the polarizing microscope. On the other hand, the connective tissue of the fibrous capsule and of the trabecular strands separating the tumor cell nests, when undergoing lipoid alterations and transformation, has not doubly refractive properties. In other tumors, like fibromas and papillomas, the doubly refractive droplets filling leukocytes within the small vessels are frequently the chief or even the only lipoids present in the tissue outside the epithelial layer.

A considerable amount of lipoids was also found in a hard or composite odontome. The main substance of this tumor, which was as large as a walnut, was dentin; enamel was present almost exclusively on the outer surface in the form of nodules and scales. Microscopically the dentin was arranged either in irregular solid conglomerations or, more generally, in layers around spaces which represented the pulp chambers. Some of the spaces were filled with cellular tissue resembling that of the pulp, others were occupied, partially or totally, with a more or less homogenous material. Some were empty. A noticeable feature of sections from the tumor in regard to the lipoids therein, was that these substances were almost throughout limited to the regions where there were still some cellular elements, namely to the pulp spaces, and to the dentinal tubules. They occurred in fine granules which stained orange red by Sudan III and purplish by Nile blue. In many regions the tubules of the dentin were filled with such granules. Sometimes a direct connection of these with the lipoids of the pulp spaces was plainly visible. Most of these substances were cholesterolesters or mixtures of cholesterol with fatty acids. There was practically no neutral fat present, and very little of those substances which react to Fischler's staining method for fatty acids and soaps.

In a series of tumors the chief lipoids, as far as the staining methods enable us to determine them, were fatty acids and soaps. These are placed in one group in my study, because their staining reactions are the same. I have made no attempt to ascertain the nature of these compounds. In Kawamura's table,

the reactions for fatty acids are in parenthesis referred to oleic acid, and those for soap, to sodium oleate. Such lipoid substances were found within the squamous epithelial covering of papillomas, fibromas, and the mucosa of ordinary hypertrophy of the gum. They were, however, by no means found in all such specimens. The great irregularity in the amount, locality, and distribution of these lipoids is a notable feature; thus it has happened that of twenty sections nineteen were absolutely negative and the only positive one showed a relatively large amount of fatty acid. The site of these lipoids is of interest. In such pathological specimens, the outer layer of the mucous membrane of the squamous cell covering of tumors may present alterations which resemble cornification of the epidermal layer. Such areas take a rosy tint with Sudan III, stain intensely by Fischler's and Smith's methods, and are positive by Ciaccio's. But deeper portions, also, and the innermost layer of the epithelial "pearls," may be distinctly positive with Fischler's, the specific stain for fatty acids and soaps. This finding illustrates well the close relationship of the mucous membrane to the skin, in which lipoids, or "fat," as they have habitually been called, have been found by writers like Unna (11), Sata (12), Karwicka (13) and others. Unna demonstrated a "fat impregnation" in the horny layer of the normal skin. Sata found "fat" chiefly in the rete malpighii. Both reported also the presence of fat in the parietal cells of the vessels of the skin, as well as between the red corpuscles and within lymph-spaces and lymph-vessels. Similar observations have been reported by others. A relationship of eleidin to fatty substances has been suggested. There are others, however, who dispute Unna's assertions. Hagemeister even declares that cornification and fat infiltration exclude one another. He states that this form of necrosis is incompatible with fat-formation.

The largest amount of lipoids demonstrable by Fischler's method was found in papillomas (figs. 2 and 3), and here they were present almost to the exclusion of all other lipoids. They stain deeply blue or almost black, by Fischler's and Smith's methods, and are pinkish with Sudan III. Under the polarizing micro-

scope they appear as most brilliant silver masses. In no instances are doubly refractive droplets observed. Unna called such infiltration in the skin "fat impregnation." Since we know now that the impregnating substance is not neutral fat, "lipoid impregnation" is probably the better name. It is worth mentioning that in the process of being stained by Ciaccio's method,

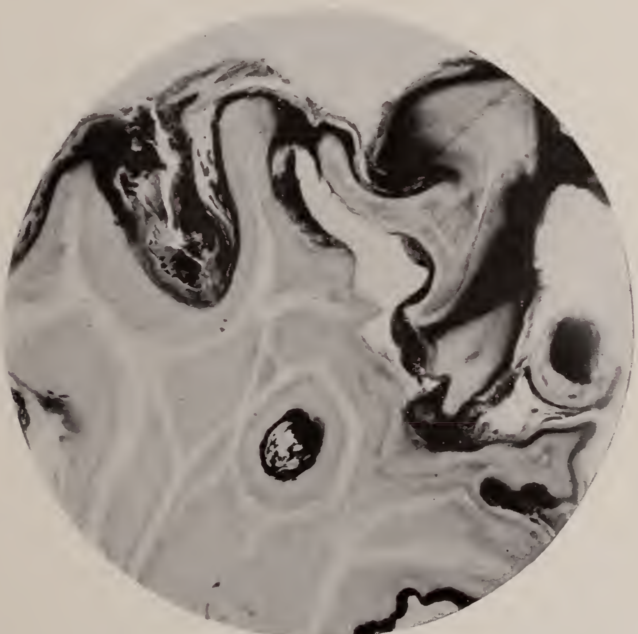


FIG. 2. SECTION PREPARED BY SMITH'S METHOD

From a papilloma, the size of a pea. The black masses, which are dark blue and black in the stained section, represent fatty acids and soaps, all strictly limited to the layer of squamous epithelial cells. × 40.

the regions giving a positive Smith and Fischler reaction take a pink hue with Sudan III, which, however, is crowded out by the subsequent staining with Heidenhain's iron hematoxylin, so that such regions appear finally stained dark blue. Substances giving the same reactions to Fischler's and Smith's methods, to Nile blue, and to Sudan III, and showing the same characteris-

tics under crossed Nicols, were found in tumors without squamous epithelial covering, namely in the walls of vessels within the connective tissue capsule (fig. 4). Hyaline degeneration, so called "fatty degeneration," and calcification were frequently observed vascular alterations. Hyaline degeneration and lipoid "impregnation" were also found on a large scale in tumors about

Fig. 3. Section Prepared by Fischler's Method

From a fibroma. The black masses are dark blue in the stained section; the lipoid substances (fatty acids and soaps) in this region occupy all the layers of the squamous epithelial covering. × 40.

slits and spaces filled with cholesterol crystals. It would seem that these degenerative changes associated with the occurrence of fatty acids and soaps are in some way, directly or indirectly, connected with calcium deposition. But generally accepted views concerning the processes involved in calcification are still lacking. There is not yet any final explanation of just why cer-

tain hyaline degenerated tissues bind calcium. Fatty acids do not normally occur in tissues as such. Pathologically, however, they are observed in necrotic foci, especially of the lung, of the lipoid tissue of the pancreas, of abscesses, and of tumors. Calcium soaps, according to Aschoff, are being more frequently

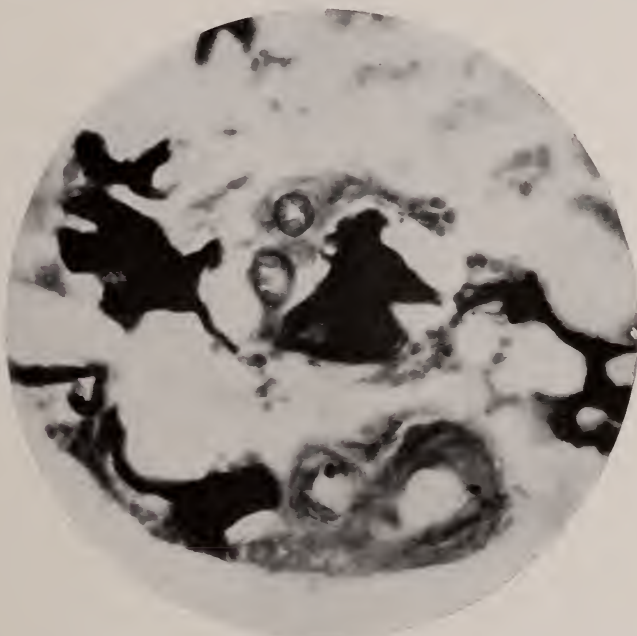


FIG. 4. SECTION PREPARED BY CIACCIO'S METHOD AND STAINED WITH NILE BLUE

From the thick connective tissue capsule of an angioendothelioma, the bundles of which are separated and the spaces occupied by small thick-walled vessels and blood effusions; the black areas are homogeneous masses stained blue in the section and represent the degenerated blood vessels (fatty acids). Four well preserved small vessels are in the same field. × 475.

observed. They occur in arteriosclerosis, in the border zone of infarcts of the kidney, in caseous regions, and, in short, wherever processes lead to calcification. Calcium deposition is not uncommon in tumors. It is observed especially in tumors of the endothelial and epithelial cell type, and here it occurs not only in concentrically placed dead endothelial cells, in cornifying and colloid degenerated cells, but also in the hyaline substance ex-

creted by the cell. Aschoff (14) refers to a close relationship of hyaline and colloid substances to fibrin, which fact seems to explain, he states, why fibrinous hyaline masses, resulting from simple coagulation and coagulation necrosis, degenerated epithelial cells, and hyaline degenerated fibrous intercellular tissue, take up calcium with such readiness.

FATTY ACIDS AS A FACTOR IN CALCIFICATION

I have not entered into an investigation of the nature of soaps and fatty acids in tumors and their relationship to calcification, this being a question beyond the scope of this paper. Although it seems to suggest itself that the same areas which present degenerative changes associated with lipoid deposition may at the same time be the seat of calcareous infiltration, because of the presence of these lipoid substances, the methods which I employed are not the same to prove or disprove any direct relationship of the lipoids to calcification. The opinions concerning this question are divided. Klotz (15) and Aschoff (14) believe that the fatty acids are the "Kalkfänger," i.e., substances which bind calcium from the blood, and that the formation of such soaps is the stage preceding calcification. Subsequently the fatty acids may be replaced by carbonic and phosphoric acids. It is generally admitted that this is the manner in which calcification takes place in fat necrosis of the pancreas and of lipomas. Whether such a process also plays a part in calcification otherwise is not yet determined. Wells (16) denies it. From the results of chemical analyses of normal and pathological material, he came to the conclusion that fatty acids are seemingly not an important factor in the binding of calcium in pathological calcification, and that there is no evidence that calcium soaps form a constant and important stage in the process of calcification. Degeneration and the presence of lipoids, however, are prominent features in areas which are to become calcified. An essential difference between pathological calcification and normal ossification is that in the former the vitality of the tissue is lowered or totally lost and that therefore any cell or tissue may be involved, provided it has degenerated sufficiently, while ossifica-

tion is accomplished only in varieties of connective tissue. In calcification, the significance of the presence of lipoids in degenerated areas is illustrated by Wells, when he refers to differences in healing caseous tubercles and gummas. The former always show much fatty degeneration and almost invariably become calcified when they heal, while gummas are relatively little involved in fatty degeneration and usually do not become calcified.

SOURCE AND SIGNIFICANCE OF CHOLESTEROL

A predominant interest has been attached to the question of the source and the significance of cholesterol infiltrating the cells. Lipoids are a normal constituent of all cells. Physiologically they may be present in large quantities in the liver cells and in the suprarenals; these organs seem to play an important part in the cholesterol regulating metabolism. The adipose tissue is the chief depot in which the lipoids are stored and from which they are conveyed to other tissues in physiological and pathological conditions. In this transport by way of the blood and lymph they have not the physicochemical constitution in which they reside in the cells, but are split into their components and in the form of a solution enter the new cells, there to be reconstructed synthetically. The transmission of lipoids by the blood or lymph may be said to have been conclusively demonstrated by experimental investigations, chiefly those of Rosenfeld (17). Hagemeister (18), on the other hand, claims to have evidence that the source is local. According to him lipoids are liberated from the infiltrated cells in the process of tissue necrosis, and are taken up by the transudate, through which they are imparted to neighboring cells. The transmission is entirely local. It will be admitted that unquestionably such conditions may take place; cells may take up lipoid substances directly from their environment; von Recklinghausen (19), Beneke (20), and Arnold (21), have shown this by animal experiments. Also histological studies of emboli, encephalitic foci, give evidence of a direct intake. But there is nothing to prove that this is the only one or is even frequent. Hagemeister's theories as to the significance of cholesterol and the lipoids are not generally

shared. He believes he has demonstrated that lipoids are found only and regularly when there is necrosis; he goes a step further and states that "fat" may be taken up into dead cells, and that on the other hand, increased cellular activity, hyperemia, and proliferation are even instrumental in causing the disappearance of lipoids. These observations have not been confirmed by other investigators. In fact, White states that the cholesterol combinations which he studied by the polarizing microscope occur chiefly in the proliferating areas of tumors. According to him, this location is significant. As cholesterol normally and physiologically plays an important part in the economy and is by no means a waste product, so in tumors it may have an important function to perform. The peculiar location would suggest that it may, in some way or other, be associated with the regulation of proliferation. I may remark here that Hagemeister's failure to observe lipoids in localities where others have found them is most probably due to the staining method he employed. Osmic acid, which he used exclusively, is a stain which by no means uncovers all lipoid substances. As is well known, it is reduced only by oleic acid, olein, and so-called lecithin. Some of his deductions must therefore be regarded as disputable, because they are made from incomplete observations. Contrary to his views lipoid deposition in cells is considered to be an entirely vital process, although its presence is often a sign of existing degenerative alterations. In tumors such as carcinoma, sarcoma, lymphosarcoma, and adenoma, it is often associated with regressive changes, but is not found in the necrotic areas. Anisotropic droplets do not occur in dead cells. Lipoids may be found in cells without nuclei, but they were there before the death of the cell occurred.

According to Aschoff (4) the storing of lipoid substances by cells in the neighborhood of dying tissue, especially in tumors and certain abscesses, is nothing else but a process of resorption of doubly refractive substances which have been set free. There may be marked degenerative processes in cells infiltrated with cholesterol, but the very presence of this substance is a sign that they are still alive, and still active. It also indicates that it is not a mere waste product, but may be a substance involved

in some active function. Rosenfeld even expresses the view that it is of a regenerative character.

The occurrence of these substances is apparently dependent on the blood supply. A characteristic feature of the infiltration in the liver, for example, is the arrangement of the anisotropic droplets along that side of the liver cells which is nearest to the blood-capillaries. Local anemia leads to the so-called fatty degeneration; the cholesterol mixtures, however, are found only in those regions where there are still some tissue fluids circulating, for example, in infarcts within the outer zone. In tubercles, also, they are not in the central region of necrosis, but in the peripheral zone of epithelioid cells. Even in the giant cell the homogeneous protoplasmic portion is free from cholesterol. The lipoid is found only about the peripherally placed nuclei. The analogy in the arrangement of the lipoid substances in the giant cell and the tubercle is interpreted by Herxheimer (22) to indicate that the central portion free from nuclei in the giant cell, may be considered as undergoing necrosis.

The presence of cholesterol, therefore, far from being a sign of disease, denotes life and activity. Cells very seriously injured, although still alive, seem to be unable to store lipoids.

SUMMARY

1. The selective staining methods of Ciaccio, Smith, and Fischler, used with the general lipoid stains, Sudan III and Nile blue, and reinforced by the aid of the polarizing microscope, are fairly adequate means for a histomorphological differentiation of the various lipoids present in tissues.

2. The lipoids in pathological dental tissue are chiefly cholesterol, occurring as the stable ester compound, but much more frequently in more or less loose combinations with fatty acids and other lipoids. The fatty acids and soaps which are found in certain tumors, are located chiefly in the zone of squamous epithelial cells. Otherwise, they occur in the degenerated walls of blood-vessels in tissue with a depleted blood supply.

3. The mixtures of cholesterol with fatty acids occur in the form of droplets and granules within cells, chiefly endothelial

cells and leukocytes. They have anisotropic properties. There are mixtures of cholesterol with glycerinesters and probably other lipoids in degenerated connective tissue which have not the form of droplets and granules and are not doubly refractive.

4. The lipoids in pigments seem to be chiefly mixtures of cholesterol with glycerinesters. Double refraction is not observed in pigments.

5. Fatty acids and soaps are found in areas where hyaline degeneration and calcification also are observed. The question of whether, or how, these substances may be involved in the process of calcium deposition has not been approached in the practical part of this study.

REFERENCES

(1) White: Jour. Path. and Bacteriol., 1909, xiii, 4.
(2) Ciaccio: Centralbl. f. allg. Path. u. path. Anat., 1909, xx, 771.
(3) Podwyssotzky: Grundzüge der allgemeinen Pathologie, 2nd edition, 1898, v. 1 (abstr. in Lubarsch-Ostertag, Ergebn. d. allg. Path., 1898, v. 713.
(4) Aschoff: Zieglers Beitr., 1910, xlvii, 1.
(5) Kaiserling: Berl. klin. Wchnschr., 1910, xlvii, 2156.
(6) Kawamura: Die Cholesterolverfettung, Jena, 1911.
(7) Bell: Jour. Med. Research, 1911, xix, 539.
(8) Smith: Jour. Path. and Bacteriol., 1908, xii, 1.
Smith and Mair, ibid., p. 134.
(9) Dietrich: Verhandl. d. deutsch. path. Gesellsch., 1910, xiv, 263.
(10) Fischler: Centralbl. f. allg. Path. u. path. Anat., 1904, xv, 1913.
(11) Unna: Monatschr. f. prakt. Dermat., 1898, xvi, 601.
(12) Sata: Zieglers Beitr., 1900, xxvii, 555.
(13) Karwicka: Virchows Arch., 1906, clxxxiv, 414.
(14) Aschoff: Lubarsch-Ostertag, Ergebn. d. allg. Path., 1902, viii,[1] 561.
(15) Klotz: Jour. Exper. Med., 1905, vii, 633.
(16) Wells: Jour. Med. Research, 1906, N.S. ix, 491.
Wells and Benson: ibid., 1907, N.S. xii, 15.
Wells and Mitchel: ibid., 1910, N.S. xvii, 501; Arch. Int. Med., 1911, vii, 721.
(17) Rosenfeld: Verhandl. d. Cong. f. inn. Med., 1897, xv, 427.
(18) Hagemeister: Virchows Arch., 1903, clxxii, 72.
(19) von Recklinghausen: Handbuch der allgemeinen Pathologie des Kreislaufes und der Ernährung, 1883, chapter xv.
(20) Beneke: Zieglers Beitr., 1897, xxii, 342.
(21) Arnold: Virchows Arch., 1903, clxxi, 197; Centralbl. f. allg. Path. u. path. Anat., 1905, xvi, 481.
(22) Herxheimer: Lubarsch-Ostertag, Ergebn. d. allg. Path., 1902, viii,[1] 681.

THE SIZE OF THE SPLEEN IN IMMUNE MICE[1]

WILLIAM H. WOGLOM

From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director

Received for publication, February 11, 1919

HISTORICAL REVIEW

It has been asserted by not a few authors in recent years that the spleen is enlarged in mice bearing, or immune to propagable tumors; and implied, if not actually stated, that the hypertrophy is an expression of the important part taken by this organ in the elaboration of protective substances of all sorts. This involves the subsidiary assumption that immunity to transplantable new growths is due to some sort of antibody, though it is admitted that no evidence of the presence of any antibody similar to those active against the various bacteria has yet been advanced.

Cimoroni (1), Brancati (2), Price-Jones (3), and Mazza (4), have all recorded enlarged spleens in mice or rats bearing transplantable tumors. Thus Brancati wrote that whereas in the normal animal the relation of spleen weight to body weight is as 1 to 169, in those with tumors it is as 1 to 60. Mazza asserted that the spleen is enlarged in rats developing small multiple nodules after intraperitoneal inoculation, but of normal size in those with a single large growth. Price-Jones found that the two heaviest spleens in his series occurred in the two mice with the largest tumors, and that there was about twice as much myeloid activity going on in the spleens of cancer mice as in those of normal ones. In a later paper (5) he described a group

[1] A preliminary report of these investigations was presented at the Tenth Annual Meeting of the American Association for Cancer Research, New York, April 5, 1917. See Jour. Cancer Research, 1917, ii, 501.

of rats bearing transplantable sarcoma. The spleen had increased 56 per cent in weight in rats with non-ulcerated tumors, 115 per cent in the animals whose tumors had undergone slight ulceration, and 130 per cent in those with extensive ulceration. As there were only two rats with non-ulcerated growths, he left open the question whether the spleen is enlarged in animals bearing non-ulcerated neoplasms.

Apolant (6), on the contrary, declared definitely that the splenic enlargement in tumor-bearing mice is not due to ulceration, for he found that in animals with old and deeply ulcerated neoplasms (especially sarcomata) the spleen is extraordinarily small. Apolant asserted, furthermore, that this organ is enlarged not only in mice bearing spontaneous or transplanted growths, but also in those that have been unsuccessfully inoculated with tumor, or immunized with normal tissue.

A second reason for crediting the spleen with an active rôle in protecting the body against transplanted cancer has been the observation, frequently recorded by pathologists, that metastases are rarely found in this organ. Still, there is good reason to believe that this is not solely because it offers a soil antagonistic to the growth of the cancer cell, but because the cancer cell often does not find itself amid suitable mechanical conditions. Within the past few years v. Hansemann (7) has emphasized his belief that metastases are not so rare in the spleen as they are ordinarily supposed to be, and Kettle (8) has ascribed what freedom the spleen does enjoy, if any, to the contractions of this organ, which force tumor emboli out of it or prevent the growth of such as have already succeeded in becoming impacted in its capillaries. Finally, the argument is clinched by the observation of Graf (9), Goldmann (10), and Endler (11), that in animals tumor grafts grow after having been inoculated into the spleen. In the experiments of Graf, indeed, 84 per cent of such grafts were positive, an outcome that compares favorably with the result following subcutaneous transplantation. Even Brancati (12), a believer in the immunological importance of the spleen, admits that tumor grafts will grow in this organ, though it is true that he says the proliferation is more restricted than at other sites.

A third reason that has been advanced to uphold the importance of the spleen as a protective factor is the assertion of Apolant that the resistance of an immune animal can be abrogated by splenectomy (13), and that it is difficult to immunize splenectomized animals; the fact that they can be immunized at all, however, suggested to him that the spleen cannot be the only organ involved in the elaboration of resistance. Both these statements have been challenged by Bullock and Rohdenburg (14), who found that removal of the spleen does not interrupt an existing immunity, and that splenectomized mice are made resistant as easily as any others.

A fourth claim has been entered for the spleen, in the assertion that tumors grow more readily in splenectomized animals because of the removal of its protective action (Oser and Pribram (15); Brancati (16)). But Rohdenburg, Bullock, and Johnston (17), Biach and Weltmann (18), Morris (19), and Mottram and Russ (20), have all found that splenectomy neither increases nor decreases the receptivity of normal animals for tumor grafts. Mottram and Russ say, however, that when immune rats are splenectomized, some microscopic growth of the tumor results. With the idea that any increase in susceptibility, however slight, would be best shown after inoculation with a tumor giving a low percentage of takes, Bullock and Rohdenburg (21) transplanted spontaneous mouse tumors into a series of splenectomized mice, but found them no more receptive than normal controls.

Finally, as a fifth argument for the participation of the spleen in the immune reaction, it has been asserted that treatment of tumor-bearing animals with the spleens of mice that have been intraperitoneally or subcutaneously injected with tumor will bring about a recession of their growths (22). The explanation of this statement lies in Braunstein's naive remark, that the results seem to have been better with sarcoma; for it is now well known that transplantable sarcomata regress without any treatment in a large percentage of the animals bearing them. Furthermore, Morris (23), who has but recently investigated this question with great care and on a large series of animals,

was quite unable to discover the slightest effect upon transplantable mouse sarcoma after injecting the animals with the spleen or blood of normal, tumor-bearing, or immune mice.

A closely related assertion is that of Mottram and Russ (24), and Biach and Weltmann (25), that spleen will inhibit the taking of a rat sarcoma where it is mixed with the tumor before inoculation. Both papers contain the statement that other organs will not do this, and that the effect is especially distinct when spleen from tumor-bearing rats is employed, invoking as an explanation the presence in this tissue of some specific substance able to damage the tumor cell. Mottram and Russ, also, seem somewhat inclined to accept a similar explanation; at any rate, they say that the result is not to be ascribed to any general immunizing action exerted by the spleen, for when tumor is inoculated on one side of the animal and spleen on the other, no effect is observed. These latter authors describe, also, a great increase in the total number of lymphocytes and plasma cells in the spleens of most immune rats, a reaction which they compare with that described by Da Fano (26) in the subcutaneous tissues.

The preceding brief review of the question shows that almost no evidence has yet been advanced for the significance of the spleen in resistance that cannot be matched with equally good evidence against its participation; so many are the unknown factors in the equation which the investigator of cancer is called upon to solve.

CAUSES OF ENLARGEMENT

It is not the purpose of the present paper to deny that the spleen has any part in the production of the refractory state, but only to show that there is, so far, no incontrovertible proof that it has; and, particularly, to demonstrate that enlargement of the spleen in mice does not prove that this organ participates in bringing about resistance to the inoculation of a tumor.

Underlying all the reports of an hypertrophied spleen in tumor-bearing or immune mice is the tacit assumption that the organ was of normal size before inoculation. But this supposition is quite unwarranted, for the spleen is frequently enlarged

in mice that have received neither an immunizing injection nor a tumor graft, being sometimes eight times the normal size (27). In the white rat, too, it may often be several times the normal weight (28). Yet only a few investigators appear to have been aware of this, though the wide differences in so-called normal animals were described some years ago by Medigreceanu (29). Because of its variable size, Medigreceanu was unwilling to say whether or not the spleen shares in the enlargement which he discovered to involve other organs (heart, liver, and sometimes the kidney) of tumor-bearing mice. If it does, its increase may be only a part of the general hypertrophy, and may actually help to provide for, and not antagonize the tumor, as Bashford (30) has pointed out.

Continuing Medigreceanu's investigation, Bashford has shown that hypertrophy of the spleen, if any, is greater in animals with progressively growing neoplasms than in those where the growth has been absorbed, so that the enlargement must be independent of the resistance. Finally, Rondoni (31) has insisted that hypertrophy of the spleen in tumor-bearing animals is not to be referred to any protective action exerted by this organ.

To what, then, may it be ascribed? In the mouse, at least, often to mouse typhoid. Many years ago Loeffler (32) found the spleen almost constantly enlarged in this disease, and the writer can testify to the wide distribution of mouse typhoid among white mice, in the vicinity of New York at any rate. A spleen of normal size and healthy pink color is, in consequence, rather a rare finding in adult mice, most of those examined being swollen and of a purplish hue. Furthermore, E. G. Cary of this laboratory, after an investigation of mouse typhoid and possible methods of controlling it, came to the conclusion that the majority of mice have become infected even before they are shipped from the breeding places, that is, before reaching the age of two or three months, and that stock from every dealer, within a hundred miles of New York City at least, will contain infected animals at one time or another. I wish at this point to thank Dr. Cary for his kindness in allowing me thus to refer to his still unpublished work.

The ubiquity of mouse typhoid makes it evident that statements describing enlargement of the spleen in tumor-bearing or immune mice can no longer be accepted as valid unless the size of the spleen before inoculation was known.

NORMAL SIZE OF SPLEEN

The normal volume of the spleen is unknown, as has already been intimated, but it is in the neighborhood of 0.1 cc. The authority for this supposition rests upon measurements made on five mice, three of which had been kept isolated, each in a cage alone, since they were weaned, so that the chances of infection were reduced to a minimum. In these three mice, which were eight, nine, and twenty months old respectively, the measurements were: $1.4 \times 0.35 \times 0.1 = 0.049$ cc., $1.45 \times 0.45 \times 0.15 = 0.098$ cc., and $1.7 \times 0.5 \times 0.15 = 0.127$ cc. These volumes correspond closely enough with those for the remaining two of the five, young adults which, though they had not been isolated, were found to have spleens of healthy appearance as contrasted with the swollen purplish look so often encountered. Here the dimensions were $1.3 \times 0.45 \times 0.1 = 0.058$ cc., and $1.7 \times 0.55 \times 0.17 = 0.159$ cc. The average of the five volumes is 0.098 cc.

METHOD OF MEASUREMENT

The remainder of this paper will describe a series of experiments in which the size of the spleen was known before immunization or tumor inoculation. It was thought at first that a fairly exact idea of the dimensions of the organ might be had by measurement through the shaved and wetted skin; but when figures so gained were compared with the results of direct measurement immediately afterward, the mouse having been killed in the meantime, it was discovered that the error was so high as to demand direct measurement of the spleen by laparotomy; all the figures employed are accordingly the product of this latter method.

Under ether, an incision about 1 cm. in length was made,

through which the spleen was drawn. The length, breadth, and thickness of the organ were then measured in centimeters with a slide caliper to the nearest 0.05 cm., the spleen was replaced in the abdomen, and the wound repaired with one continuous suture. Owing to the curvature of the spleen, the length as measured was somewhat less than the actual length; but the organ was laid as flat as possible on the abdomen, and the error was about the same in each mouse, so that the results are fairly comparable. The method is not accurate in any case, for the breadth and thickness vary somewhat in any one spleen, and all that can be done is to select what appears to be the average dimensions; it is unfortunate that the nature of the experiment made it impossible to employ the weight of the organ, rather than its volume.

SOURCES OF ERROR

In measuring a yielding structure like the spleen, it is obvious that there will be some personal error. To determine its magnitude, a dozen mice were killed and all twelve spleens measured once in their three dimensions. The organs were then measured a second time and the results recorded on another slip of paper; the length of time consumed by the first measurement (twenty minutes) and the number of dimensions recorded (thirty-six) precludes the possibility that any of the results were remembered when the second series of measurements was made. In six of the cases the second measurement was larger, in five smaller, and in one the two results coincided. The greatest positive error (second measurement larger) was 44 per cent, and the greatest negative was 20 per cent. An error of 44 per cent, however, is really smaller than it seems, as attention to the six measurements which produced it will show; thus the results of a first measurement were: $2.0 \times 0.6 \times 0.15 = 0.180$ ccm., and of a second: $2.0 \times 0.65 \times 0.2 = 0.260$ ccm. A difference of 0.05 cm. in each of two diameters was therefore sufficient to bring it about. Still, if the error happened once in twelve cases it might have occurred about eight times in a hundred, and hence it cannot be disregarded. It will accordingly be best to acknowledge frankly

that the personal error in all the following measurements is, in round numbers, 50 per cent and to exclude from the number of enlarged spleens all such as have not increased more than 50 per cent in volume. As the augmentation is often over 500 per cent the elimination of spleens with less than 50 per cent enlargement is not such a radical excision as it might appear to be at first sight.

In order to be sure that measurements of the spleen made during life at laparotomy are comparable with those made after death, the spleens of two mice were measured under ether anaesthesia. The animals were then killed and the spleens measured again about fifteen minutes after death. No difference was found, and it is a legitimate conclusion, therefore, that the post mortem measurements in this paper, all of which were made within fifteen or twenty minutes after death, may be compared or contrasted with others made during life.

But an essential preliminary to any such procedure is a knowledge of whether the spleen in mice subjected to laparotomy and measurement of this organ, and otherwise untreated, will enlarge during the subsequent few weeks as a result of the operation or of other causes inseparable from the conditions governing the experiment.

Table 1 represents the survivors of thirty-six mice whose spleens were measured on December 13, 1918. These thirty animals were killed, and their spleens were measured again, two weeks later, on December 27, 1918. As in tables 2 and 3, the upper figures for each mouse give the volume in cubic centimeters at the first measurement, while the lower are the volume at autopsy. The percentage is the percentage of enlargement; where it has been omitted, the spleen at autopsy was the same size as at the first measurement, or smaller. In the latter case, the organ is to be regarded merely as not having been enlarged, consideration of its shrinkage lying outside the domain of the present discussion.

Of the thirty mice in the table, ten had spleens that were not enlarged at autopsy. In the remaining twenty, six showed an enlargement exceeding the personal error of 50 per cent. There-

fore, of a group of mice laparotomized and set aside for two weeks, about 20 per cent developed an hypertrophy of the spleen.

In a second experiment (table 2), a longer period was allowed to elapse between the first and second measurements. The spleens of thirty-six mice were measured on April 28, 1916, and

TABLE 1

MOUSE	VOLUME OF SPLEEN	ENLARGEMENT	MOUSE	VOLUME OF SPLEEN	ENLARGEMENT	MOUSE	VOLUME OF SPLEEN	ENLARGEMENT
	cc.	per cent		cc.	per cent		cc.	per cent
1	0.108 0.120	11	13	0.187 0.222	19	25	0.187 0.195	4
2	0.101 0.170	68	14	0.112 0.148	32	27	0.144 0.153	6
3	0.127 0.190	50	15	0.101 0.142	142	28	0.153 0.131	
6	0.216 0.116		16	0.148 0.076		29	0.112 0.260	132
7	0.204 0.198		17	0.105 0.170	62	30	0.120 0.072	
9	0.132 0.160	21	18	0.094 0.101	7	31	0.176 0.116	
10	0.058 0.127	119	20	0.162 0.127		32	0.160 0.160	
11	0.090 0.131	45	21	0.198 0.240	21	33	0.200 0.210	5
12	0.127 0.102		22	0.094 0.198	111	34	0.132 0.170	29
			23	0.170 0.214	26	36	0.432 0.135	
			24	0.198 0.214	8			

Mice laparotomized and set aside for two weeks. About 20 per cent have developed an hypertrophy of the spleen.

the animals were killed on June 14, 1916—forty-seven days later. In seven of the thirty survivors, the spleen had not enlarged. Of the remaining twenty-three, fourteen showed an enlargement of more than 50 per cent. Therefore, of a group of mice laparotomized and set aside for about seven weeks, approximately 50 per cent developed an hypertrophy of the spleen. Not only

TABLE 2

MOUSE	VOLUME OF SPLEEN	ENLARGEMENT	MOUSE	VOLUME OF SPLEEN	ENLARGEMENT	MOUSE	VOLUME OF SPLEEN	ENLARGEMENT
	cc.	*per cent*		*cc.*	*per cent*		*cc.*	*per cent*
1	0.136 0.190	40	14	0.096 0.252	162	27	0.166 0.367	121
2	0.116 0.127	9	15	0.121 0.064		28	0.124 0.132	6
3	0.093 0.092		16	0.090 0.121	34	30	0.228 0.308	35
4	0.108 0.252	133	17	0.049 0.200	308	32	0.073 0.200	174
5	0.054 0.038		18	0.175 0.135		33	0.042 0.153	264
6	0.048 0.087	81	19	0.093 0.190	104	34	0.294 0.308	5
7	0.252 0.118		20	0.090 0.102	13	35	0.040 0.132	230
8	0.127 0.294	131	21	0.038 0.036		36	0.116 0.220	90
10	0.200 0.264	32	22	0.058 0.090	55			
11	0.013 0.120	823	23	0.228 0.231	1			
12	0.060 0.264	340	24	0.140 0.124				

Mice laparotomized and set aside for nearly seven weeks. About 50 per cent have developed an hypertrophy of the spleen.

does the number of mice so affected increase with time, but the enlargement is greater. Thus, the largest spleen in this series had undergone an increase of 823 per cent, as compared with 142 per cent after two weeks. It cannot be objected that in the latter instance the organ had reached dimensions beyond which it is impossible to increase, for its final volume was but 0.142 cc., whereas spleens over 0.300 cc. in volume can be found in both tables.

It is impossible to prove that the hypertrophy in the two preceding experiments was due to cage infection rather than to infection at operation, but the following facts strongly support the view. The mice in tables 1 and 2 are arranged according to their disposition in boxes; column 1 in each table contains survivors of the twelve mice of Box I, column 2, those of Box II, and so on. The individual mice are numbered in the order in which they were operated upon, the missing numbers, of course, representing those that died before the experiment was terminated. The mice in Box I were always laparotomized in the morning, and those of Boxes II and III at one sitting in the afternoon, the instruments being sterilized when Box I was begun, and once again for both Boxes II and III. Immersion in alcohol was relied upon to keep the instruments reasonably clean between individual operations. Now if infection at operation were the cause of the splenic enlargement, it should become increasingly evident in the higher numbers of Boxes I and II, and should be greatest in Box III, since the instruments had been used on twelve mice before this box was begun. Yet in table 1, only one of the ten mice in Box III (last column on right) had a spleen enlarged more than 50 per cent of its size at the first measurement. Again, the three mice that died in Box I were not Nos. 10, 11, and 12, as might have been expected had infection played any considerable part in their subsequent history, but Nos. 4, 5, and 8. Similarly in Boxes II and III, the last mice to be operated were not the ones that died (with the single exception of No. 35 in Box III). A similar discrepancy between the chances of operative infection and the occurrence of death is to be noted in table 2; nor is there any greater tend-

ency here than in table 1 for the mice last operated upon to have large spleens. It is unnecessary to labor the point, for in no way can a positive proof be adduced from the data at hand, but it seems fair to conclude that laparotomy does not result in enlargement of the spleen, when done with reasonable cleanliness.

Infection with the *B. typhi murium*, on the other hand, will cause the spleen to hypertrophy, as table 3 demonstrates. A group of mice with measured spleens were inoculated with an organism recovered at this laboratory. Except for an isolated case (mouse 4), there appears to be little or no increase in the volume of the spleen before the eighth day, where the dose was 0.05 cc. of a two-loop emulsion. At this time, some were enlarged over 50 per cent of their volume and some were not, but by the thirteenth day there was considerable increase in size. Where 0.1 cc. of emulsion was injected, the enlargement was greater and set in earlier.

It is quite permissible, therefore, to refer the splenic enlargement noted in table 1 to cage infection with the bacillus of mouse typhoid, since it has been proved that nearly all mice suffer from this disease, and that its bacillus will cause the spleen to hypertrophy.

Before going further, it will perhaps be advantageous to review the sources of error so far discussed. First, the normal size of the mouse spleen is not accurately known; secondly, there is a personal error in measuring this organ which may amount to about 50 per cent; thirdly, the spleens of mice kept in a cage with other mice will often undergo enlargement.

Variations in the size of the spleen brought about by its rhythmic contractions do not enter as a source of error, for they do not exceed 20 per cent of its volume, at least in the dog and cat. There is no reason to suppose that they are two and a half times as great in the mouse, and they therefore come, in all probability, well within the personal error. But even though they do not, the error is the same throughout the experiment, and, by reason of the large number of measurements, may be disregarded.

Finally, because the relation between splenic hypertrophy and

TABLE 3

	0.05 CC. OF A TWO-LOOP EMULSION				0.1 CC. OF A TWO-LOOP EMULSION		
		cc.	*per cent*			*cc.*	*per cent*
1	Died 1 day after inoculation	0.036 0.012		14	Killed 8 days after inoculation	0.390 0.550	41
2	Died 5 days after inoculation	0.135 0.132		15	Killed 8 days after inoculation	0.112 0.308	175
3	Died 5 days after inoculation	0.108 0.111	3	16	Died 8 days after inoculation	0.065 0.469	621
4	Died 5 days after inoculation	0.142 0.240	69	17	Died 11 days after inoculation	0.108 0.423	292
5	Died 8 days after inoculation	0.260 0.185		18	Died 12 days after inoculation	0.060 0.441	635
6	Killed 8 days after inoculation	0.198 0.157					
7	Died 8 days after inoculation	0.157 0.198	26				
8	Killed 8 days after inoculation	0.273 0.510	87				
9	Died 8 days after inoculation	0.148 0.329	122				
10	Killed 8 days after inoculation	0.072 0.305	324				
11	Killed 13 days after inoculation	0.127 0.252	98				
12	Killed 13 days after inoculation	0.127 0.287	126				
13	Killed 13 days after inoculation	0.209 0.564	170				

Mice infected with the bacillus of mouse typhoid. The resulting disease causes splenic hypertrophy.

ulceration appears to be still a matter of uncertainty, mice with ulcerated tumors have been uniformly excluded from these experiments.

The ground having been thus cleared, it will be possible now to approach the main question: Is the spleen enlarged in mice immune to transplantable tumors, by virtue of their immunity?

EXPERIMENTS

In the following tables (4 to 11 inclusive) the mice have been numbered in agreement with the corresponding charts (figs. 1 to 9 inclusive). The upper row for each mouse represents the three dimensions of the spleen and its volume in cubic centimeters at the first measurement, while the lower gives similar data as collected at autopsy.

Figure 1 (experiment $\frac{63}{128\,\mathrm{I}}$) reproduces 18 survivors of a group of thirty-six mice with spleens measured on March 16, 1916 (see table 4). Fifteen days later (March 31) these mice (Nos. 1 to 18) were immunized by the injection of 0.1 cc. of total embryo and placenta into the left axilla, and thirteen days after this immunizing treatment, they were inoculated with 0.02 cc. of carcinoma 63 in the opposite axilla.[2] They were killed, and their spleens were measured again on May 16—forty-six days after the immunizing injection. Reference to mice 19 to 28, normal untreated controls inoculated at the same time with an equal dose of the same tumor, will show that fourteen of the eighteen treated mice were immune to the tumor (Nos. 5 to 18). Yet of these fourteen immune mice, six, or 43 per cent, showed no hypertrophy of the spleen. Conversely, the mouse that developed the largest tumor (No. 1), and was therefore the least resistant of the entire group, had the third largest spleen in the series (341 per cent enlargement).

[2] The three propagable carcinomata used in these experiments (63, 206, and T) are from the Imperial Cancer Research Fund in London, whose Director, Dr. J. A. Murray, I wish to thank for his courtesy.

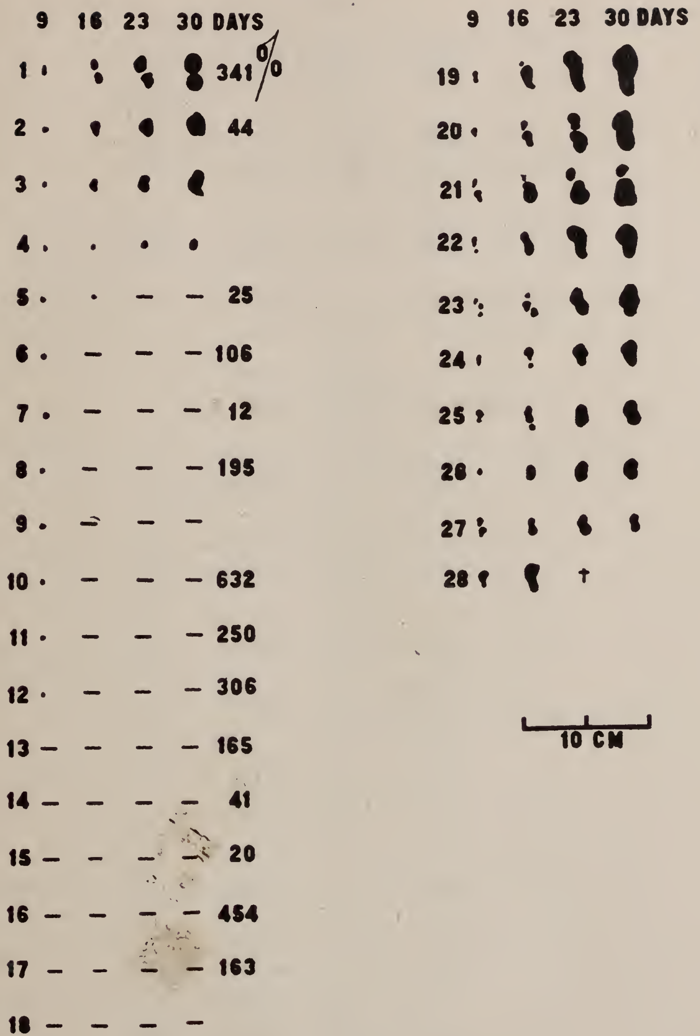


FIG. 1. EXPERIMENT $\frac{63}{128\,\mathrm{I}}$

Mice 1 to 18 had their spleens measured March 16, 1916. Immunized March 31, with 0.1 cc. total embryo and placenta. Inoculated with 0.02 cc. of emulsified carcinoma 63 April 13. Killed and spleens measured May 16, sixty-one days after the first measurement (see table 4). The mice that proved immune did not all have enlarged spleens. Mice 19 to 28 are normal untreated controls.

TABLE 4

Experiment $\frac{63}{128\ \mathrm{I}}$ (*fig. 1*)

		cc.	per cent
1	1.4 × 0.35 × 0.15 =	0.073	341
	2.3 × 0.7 × 0.2 =	0.322	
2	1.6 × 0.35 × 0.15 =	0.084	44
	1.8 × 0.45 × 0.15 =	0.121	
3	1.5 × 0.45 × 0.2 =	0.135	
	1.5 × 0.5 × 0.15 =	0.112	
4	1.7 × 0.4 × 0.15 =	0.102	
	1.5 × 0.4 × 0.15 =	0.090	
5	2.1 × 0.45 × 0.2 =	0.189	25
	2.15 × 0.55 × 0.2 =	0.236	
6	1.6 × 0.45 × 0.2 =	0.144	106
	1.7 × 0.7 × 0.25 =	0.297	
7	1.5 × 0.4 × 0.15 =	0.090	12
	1.5 × 0.45 × 0.15 =	0.101	
8	1.3 × 0.45 × 0.15 =	0.088	195
	2.0 × 0.65 × 0.2 =	0.260	
9	1.8 × 0.45 × 0.2 =	0.162	
	1.3 × 0.35 × 0.15 =	0.068	
10	1.25 × 0.35 × 0.1 =	0.044	632
	2.3 × 0.7 × 0.2 =	0.322	
11	1.4 × 0.4 × 0.15 =	0.084	250
	2.1 × 0.7 × 0.2 =	0.294	
12	1.3 × 0.35 × 0.15 =	0.068	306
	2.3 × 0.6 × 0.2 =	0.276	
13	2.0 × 0.4 × 0.15 =	0.120	165
	2.65 × 0.6 × 0.2 =	0.318	
14	1.9 × 0.6 × 0.2 =	0.228	41
	2.15 × 0.75 × 0.2 =	0.322	
15	1.6 × 0.4 × 0.15 =	0.096	20
	1.7 × 0.45 × 0.15 =	0.115	

TABLE 4—*Concluded*

					cc.	*per cent*
16	1.5	× 0.35	× 0.1	=	0.052	454
	2.4	× 0.6	× 0.2	=	0.288	
17	1.6	× 0.4	× 0.15	=	0.096	163
	2.3	× 0.55	× 0.2	=	0.253	
18	1.6	× 0.5	× 0.2	=	0.160	
	1.7	× 0.55	× 0.15	=	0.140	

No relation between splenic hypertrophy and immunity induced with normal mouse tissue.

TABLE 5

Experiment $\frac{63}{128\ \mathrm{H}}$ (*fig. 2*)

					cc.	*per cent*
1	1.55	× 0.45	× 0.15	=	0.105	99
	1.9	× 0.55	× 0.2	=	0.209	
2	1.55	× 0.5	× 0.2	=	0.155	1
	1.75	× 0.6	× 0.15	=	0.157	
3	1.7	× 0.4	× 0.15	=	0.102	
	1.55	× 0.4	× 0.1	=	0.062	
4	1.95	× 0.6	× 0.2	=	0.234	
	1.6	× 0.45	× 0.1	=	0.072	
5	1.75	× 0.55	× 0.15	=	0.144	32
	1.9	× 0.5	× 0.2	=	0.190	
6	1.3	× 0.45	× 0.15	=	0.088	10
	1.3	× 0.5	× 0.15	=	0.097	
7	1.9	× 0.55	× 0.25	=	0.261	
	1.7	× 0.55	× 0.2	=	0.187	
8	1.85	× 0.5	× 0.15	=	0.139	
	1.7	× 0.5	× 0.15	=	0.127	
9	1.8	× 0.5	× 0.2	=	0.180	
	1.1	× 0.35	× 0.1	=	0.038	
10	1.6	× 0.45	× 0.15	=	0.108	289
	2.4	× 0.7	× 0.25	=	0.420	

TABLE 5—*Concluded*

		cc.	per cent
11	1.9 × 0.5 × 0.15	0.142	86
	2.2 × 0.6 × 0.2	0.264	
12	1.9 × 0.6 × 0.25	0.285	
	1.85 × 0.55 × 0.2	0.203	
13	1.55 × 0.45 × 0.1	0.070	
	1.5 × 0.4 × 0.1	0.060	
14	1.55 × 0.4 × 0.1	0.062	211
	2.15 × 0.6 × 0.15	0.193	
15	1.6 × 0.4 × 0.1	0.064	
	1.6 × 0.4 × 0.1	0.064	
16	1.6 × 0.4 × 0.15	0.096	12
	1.6 × 0.45 × 0.15	0.108	
17	1.5 × 0.5 × 0.2	0.150	
	1.7 × 0.55 × 0.15	0.140	
18	2.2 × 0.45 × 0.2	0.198	
	2.4 × 0.55 × 0.15	0.198	

No relation between splenic hypertrophy and immunity induced with spontaneous tumors.

TABLE 6

Experiment $\frac{63}{130\ J}$ (*fig. 3*)

		cc.	per cent
1	1.3 × 0.4 × 0.15	0.078	170
	1.75 × 0.6 × 0.2	0.210	
2	1.4 × 0.4 × 0.15	0.084	183
	2.0 × 0.6 × 0.2	0.240	
3	1.5 × 0.4 × 0.15	0.090	80
	1.8 × 0.6 × 0.15	0.162	
4	1.6 × 0.4 × 0.15	0.096	30
	1.85 × 0.45 × 0.15	0.125	
5	1.65 × 0.5 × 0.15	0.124	77
	2.0 × 0.55 × 0.2	0.220	

TABLE 6—*Concluded*

					cc.	*per cent*
6	1.4	× 0.45	× 0.15	=	0.094	181
	2.2	× 0.6	× 0.2	=	0.264	
7	1.25	× 0.4	× 0.1	=	0.050	458
	2.15	× 0.65	× 0.2	=	0.279	
8	1.35	× 0.45	× 0.1	=	0.061	98
	1.8	× 0.45	× 0.15	=	0.121	
9	1.35	× 0.3	× 0.15	=	0.061	165
	1.8	× 0.6	× 0.15	=	0.162	
10	1.4	× 0.5	× 0.1	=	0.070	226
	1.9	× 0.6	× 0.2	=	0.228	
11	1.75	× 0.5	× 0.15	=	0.131	74
	1.9	× 0.6	× 0.2	=	0.228	
12	1.5	× 0.35	× 0.15	=	0.079	71
	2.0	× 0.45	× 0.15	=	0.135	
13	1.25	× 0.4	× 0.1	=	0.050	170
	1.8	× 0.5	× 0.15	=	0.135	
14	1.5	× 0.45	× 0.15	=	0.101	
	1.75	× 0.5	× 0.1	=	0.087	
15	1.1	× 0.4	× 0.1	=	0.044	139
	1.4	× 0.5	× 0.15	=	0.105	
16	1.2	× 0.35	× 0.1	=	0.042	443
	1.9	× 0.6	× 0.2	=	0.228	
17	1.1	× 0.3	× 0.1	=	0.033	127
	1.25	× 0.4	× 0.15	=	0.075	
18	1.4	× 0.4	× 0.15	=	0.084	87
	2.1	× 0.5	× 0.15	=	0.157	
19	1.25	× 0.3	× 0.15	=	0.056	171
	1.9	× 0.4	× 0.2	=	0.152	

No relation between splenic hypertrophy and immunity induced with spontaneous tumors. All spleens (except No. 11) less than 1.7 cm. in length at first measurement.

TABLE 7

Experiment $\frac{\text{T}}{\text{90 H}}$ (*fig. 5*)

		cc.	*per cent*
1	1.4 × 0.4 × 0.1 = 1.9 × 0.35 × 0.2 =	0.056 0.133	137
2	1.4 × 0.55 × 0.15 = 1.8 × 0.7 × 0.15 =	0.115 0.189	64
3	1.45 × 0.5 × 0.15 = 2.0 × 0.6 × 0.2 =	0.109 0.240	120
4	1.3 × 0.4 × 0.1 = 1.9 × 0.6 × 0.2 =	0.052 0.228	338
5	1.1 × 0.35 × 0.1 = 1.9 × 0.5 × 0.15 =	0.038 0.142	273
6	1.3 × 0.4 × 0.1 = 1.75 × 0.5 × 0.2 =	0.052 0.175	236
7	1.6 × 0.5 × 0.15 = 1.9 × 0.5 × 0.15 =	0.120 0.142	180
8	1.5 × 0.45 × 0.15 = 2.1 × 0.6 × 0.2 =	0.101 0.252	149
9	1.4 × 0.4 × 0.15 = 1.95 × 0.65 × 0.2 =	0.084 0.253	200
10	1.6 × 0.5 × 0.2 = 2.1 × 0.55 × 0.2 =	0.160 0.231	44
11	1.5 × 0.5 × 0.15 = 1.9 × 0.6 × 0.2 =	0.112 0.228	103
12	1.4 × 0.5 × 0.1 = 1.7 × 0.5 × 0.15 =	0.070 0.127	81
13	1.5 × 0.45 × 0.15 = 2.1 × 0.6 × 0.2 =	0.101 0.252	149
14	1.6 × 0.5 × 0.15 = 2.1 × 0.6 × 0.2 =	0.120 0.252	110
15	1.5 × 0.4 × 0.15 = 1.7 × 0.45 × 0.15 =	0.090 0.115	28

TABLE 7—*Concluded*

	cc.	*per cent*
16	1.5 × 0.4 × 0.15 = 0.090 1.65 × 0.45 × 0.15 = 0.111	23
17	1.6 × 0.55 × 0.2 = 0.176 1.9 × 0.55 × 0.15 = 0.157	
18	1.5 × 0.5 × 0.15 = 0.112 1.75 × 0.5 × 0.15 = 0.131	17
19	1.2 × 0.35 × 0.1 = 0.042 1.9 × 0.55 × 0.15 = 0.157	273
20	1.4 × 0.5 × 0.2 = 0.140 2.25 × 0.55 × 0.2 = 0.247	73
21	1.3 × 0.4 × 0.1 = 0.052 1.75 × 0.4 × 0.15 = 0.105	102
22	1.55 × 0.4 × 0.15 = 0.093 2.1 × 0.5 × 0.15 = 0.157	69
23	1.3 × 0.4 × 0.1 = 0.052 1.8 × 0.5 × 0.15 = 0.135	159
24	1.0 × 0.35 × 0.1 = 0.035 1.9 × 0.5 × 0.2 = 0.190	442
25	1.4 × 0.45 × 0.2 = 0.126 2.0 × 0.5 × 0.15 = 0.150	19
26	1.4 × 0.5 × 0.15 = 0.105 1.9 × 0.6 × 0.2 = 0.228	117
27	1.6 × 0.5 × 0.15 = 0.120 1.75 × 0.45 × 0.15 = 0.118	
28	1.2 × 0.45 × 0.1 = 0.054 1.7 × 0.7 × 0.15 = 0.178	229
29	1.3 × 0.4 × 0.1 = 0.052 1.8 × 0.6 × 0.15 = 0.162	211
30	1.5 × 0.4 × 0.1 = 0.060 1.8 × 0.5 × 0.15 = 0.135	125

Mice injected with non-immunizing material have enlarged spleens. All spleens under 1.7 cm. in length at first measurement.

TABLE 8

Experiment $\frac{63}{134\ T}$ (fig. 6)

	cc.	*per cent*
1	$1.35 \times 0.35 \times 0.15 = 0.071$ $1.8 \times 0.45 \times 0.15 = 0.121$	70
2	$1.2 \times 0.4 \times 0.15 = 0.072$ $1.7 \times 0.5 \times 0.15 = 0.127$	76
3	$1.5 \times 0.45 \times 0.15 = 0.101$ $1.9 \times 0.5 \times 0.15 = 0.142$	41
4	$1.2 \times 0.3 \times 0.1 = 0.036$ $1.75 \times 0.5 \times 0.15 = 0.131$	264
5	$1.4 \times 0.4 \times 0.15 = 0.084$ $2.1 \times 0.6 \times 0.2 = 0.252$	200
6	$1.3 \times 0.45 \times 0.15 = 0.088$ $1.7 \times 0.5 \times 0.15 = 0.127$	44
7	$1.3 \times 0.4 \times 0.1 = 0.052$ $1.9 \times 0.5 \times 0.2 = 0.190$	265
8	$1.2 \times 0.4 \times 0.1 = 0.048$ $1.8 \times 0.5 \times 0.15 = 0.135$	181
9	$1.25 \times 0.4 \times 0.1 = 0.050$ $1.5 \times 0.4 \times 0.15 = 0.090$	80
10	$1.3 \times 0.4 \times 0.1 = 0.052$ $1.85 \times 0.55 \times 0.2 = 0.203$	290
11	$1.5 \times 0.5 \times 0.15 = 0.112$ $1.5 \times 0.4 \times 0.15 = 0.090$	
12	$1.2 \times 0.4 \times 0.15 = 0.072$ $2.2 \times 0.7 \times 0.3 = 0.462$	542
13	$1.25 \times 0.4 \times 0.1 = 0.050$ $1.6 \times 0.5 \times 0.15 = 0.120$	140
14	$1.25 \times 0.4 \times 0.1 = 0.050$ $1.7 \times 0.5 \times 0.15 = 0.127$	154
15	$1.4 \times 0.5 \times 0.15 = 0.105$ $2.3 \times 0.6 \times 0.2 = 0.276$	163

TABLE 8—*Concluded*

					per cent
				cc.	
16	1.3	× 0.4	× 0.15	= 0.078	192
	1.9	× 0.6	× 0.2	= 0.228	
17	1.2	× 0.35	× 0.1	= 0.042	305
	1.7	× 0.5	× 0.2	= 0.170	
18	1.1	× 0.3	× 0.1	= 0.033	264
	1.6	× 0.5	× 0.15	= 0.120	
19	1.3	× 0.4	× 0.1	= 0.052	361
	2.0	× 0.6	× 0.2	= 0.240	
20	1.4	× 0.4	× 0.15	= 0.084	28
	1.6	× 0.45	× 0.15	= 0.108	
21	1.5	× 0.4	× 0.15	= 0.090	41
	1.7	× 0.5	× 0.15	= 0.127	
22	1.3	× 0.45	× 0.15	= 0.088	44
	1.7	× 0.5	× 0.15	= 0.127	
23	1.3	× 0.4	× 0.1	= 0.052	246
	1.8	× 0.5	× 0.2	= 0.180	
24	1.5	× 0.4	× 0.15	= 0.090	193
	2.2	× 0.6	× 0.2	= 0.264	
25	1.3	× 0.4	× 0.1	= 0.052	375
	1.9	× 0.65	× 0.2	= 0.247	
26	1.35	× 0.4	× 0.15	= 0.081	67
	1.8	× 0.5	× 0.15	= 0.135	

Mice injected with non-immunizing material have enlarged spleens. All spleens under 1.7 cm. in length at first measurement.

TABLE 9

Experiment $\frac{206}{177\ L}$ *and* $\frac{206}{178\ J}$ *(fig. 7)*

					cc.	per cent
1	1.1	× 0.35	× 0.05	=	0.019	1163
	2.0	× 0.6	× 0.2	=	0.240	
2	1.6	× 0.5	× 0.15	=	0.120	27
	1.7	× 0.6	× 0.15	=	0.153	
3	2.0	× 0.6	× 0.2	=	0.240	25
	2.0	× 0.75	× 0.2	=	0.300	
4	2.0	× 0.6	× 0.2	=	0.240	
	1.7	× 0.5	× 0.15	=	0.127	
5	1.7	× 0.5	× 0.2	=	0.170	
	1.7	× 0.55	× 0.15	=	0.140	
6	1.4	× 0.5	× 0.15	=	0.105	26
	1.6	× 0.55	× 0.15	=	0.132	
7	2.0	× 0.5	× 0.15	=	0.150	73
	2.0	× 0.65	× 0.2	=	0.260	
8	1.5	× 0.35	× 0.15	=	0.079	90
	2.0	× 0.5	× 0.15	=	0.150	
9	1.6	× 0.5	× 0.15	=	0.120	80
	1.8	× 0.6	× 0.2	=	0.216	
10	1.7	× 0.55	× 0.15	=	0.140	
	1.7	× 0.5	× 0.15	=	0.127	
11	1.3	× 0.5	× 0.1	=	0.065	72
	1.5	× 0.5	× 0.15	=	0.112	
12	2.0	× 0.6	× 0.2	=	0.240	8
	2.0	× 0.65	× 0.2	=	0.260	
13	1.35	× 0.4	× 0.1	=	0.054	135
	1.7	× 0.5	× 0.15	=	0.127	
14	1.6	× 0.5	× 0.15	=	0.120	
	1.6	× 0.5	× 0.15	=	0.120	
15	1.9	× 0.55	× 0.15	=	0.157	45
	1.9	× 0.6	× 0.2	=	0.228	

TABLE 9—*Concluded*

					cc.	per cent
16	1.3	× 0.5	× 0.15	=	0.097	85
	1.5	× 0.6	× 0.2	=	0.180	
17	2.0	× 0.6	× 0.2	=	0.240	
	2.1	× 0.55	× 0.2	=	0.231	
18	1.9	× 0.6	× 0.2	=	0.228	
	1.4	× 0.4	× 0.15	=	0.084	
19	1.9	× 0.65	× 0.2	=	0.247	
	1.75	× 0.45	× 0.15	=	0.118	
20	1.75	× 0.5	× 0.15	=	0.131	
	1.7	× 0.45	× 0.15	=	0.115	
21	1.3	× 0.5	× 0.15	=	0.097	123
	1.8	× 0.6	× 0.2	=	0.216	
22	1.7	× 0.5	× 0.2	=	0.170	16
	1.8	× 0.55	× 0.2	=	0.198	

No relation between splenic hypertrophy and immunity induced with transplantable tumors.

TABLE 10

Experiment $\frac{\mathrm{T}}{89\ \mathrm{I}}$ (*fig. 8*)

					cc.
1	1.7	× 0.5	× 0.2	=	0.170
2	1.7	× 0.5	× 0.2	=	0.170
3	1.8	× 0.5	× 0.15	=	0.135
4	2.4	× 0.55	× 0.2	=	0.264
5	1.8	× 0.5	× 0.2	=	0.180
6	1.75	× 0.6	× 0.2	=	0.210
7	1.7	× 0.55	× 0.2	=	0.187
8	2.4	× 0.8	× 0.25	=	0.480
9	1.8	× 0.6	× 0.2	=	0.216
10	2.1	× 0.7	× 0.3	=	0.441
11	1.8	× 0.5	× 0.2	=	0.180
12	2.0	× 0.75	× 0.25	=	0.375
13	1.8	× 0.55	× 0.2	=	0.198
14	1.75	× 0.5	× 0.2	=	0.175
15	2.0	× 0.8	× 0.2	=	0.320
16	2.2	× 0.7	× 0.25	=	0.385
17	1.8	× 0.6	× 0.2	=	0.216
18	2.1	× 0.7	× 0.2	=	0.294
19	1.9	× 0.5	× 0.3	=	0.285
20	1.5	× 0.6	× 0.2	=	0.180

No relation between splenic hypertrophy and natural immunity.

TABLE 11.

Experiment $\frac{63}{134\ Q}$ (*fig.* 9)

				cc.
1	2.3	× 0.65	× 0.25	= 0.374
2	2.1	× 0.65	× 0.2	= 0.273
3	1.7	× 0.7	× 0.2	= 0.238
4	2.1	× 0.6	× 0.2	= 0.252
5	1.6	× 0.6	× 0.2	= 0.192
6	1.75	× 0.6	× 0.2	= 0.210
7	1.7	× 0.5	× 0.2	= 0.170
8	1.55	× 0.55	× 0.25	= 0.213
9	2.1	× 0.6	× 0.2	= 0.252
10	2.2	× 0.7	× 0.25	= 0.385
11	1.7	× 0.55	× 0.15	= 0.140
12	1.7	× 0.5	× 0.15	= 0.127
13	2.0	× 0.6	× 0.2	= 0.240
14	1.7	× 0.6	× 0.15	= 0.153
15	1.8	× 0.5	× 0.2	= 0.180
16	1.75	× 0.45	× 0.2	= 0.157
17	1.8	× 0.6	× 0.2	= 0.216
18	1.9	× 0.55	× 0.15	= 0.157
19	2.0	× 0.6	× 0.25	= 0.300
20	2.2	× 0.65	× 0.2	= 0.286

No relation between splenic hypertrophy and natural immunity.

In another experiment ($\frac{63}{128\ H}$, fig. 2) spontaneous tumors were the immunizing material employed. The spleens of thirty-six mice were measured (see table 5) on March 14, 1916, and fifteen days afterward (March 29) 0.05 cc. of a mixed emulsion of seven spontaneous tumors was deposited in the left axilla. Fifteen days following immunization (April 13) 0.02 cc. of carcinoma 63 was inoculated in the right axilla, and the survivors (mice 1 to 18) were killed and their spleens measured thirty-four days after immunization (May 2). While the degree of immunity was not so high as in the first experiment, seven of the eighteen (Nos. 12 to 18) were undoubtedly immune. Yet in six (86 per cent) of these seven refractory animals the spleen had not enlarged. Mice 19 to 28 are normal untreated controls.

Since it was not known whether a period of from thirty-four to forty-six days suffices for the spleen to enlarge, the experiment was repeated ($\frac{63}{130\ J}$, fig. 3) with a longer interval between

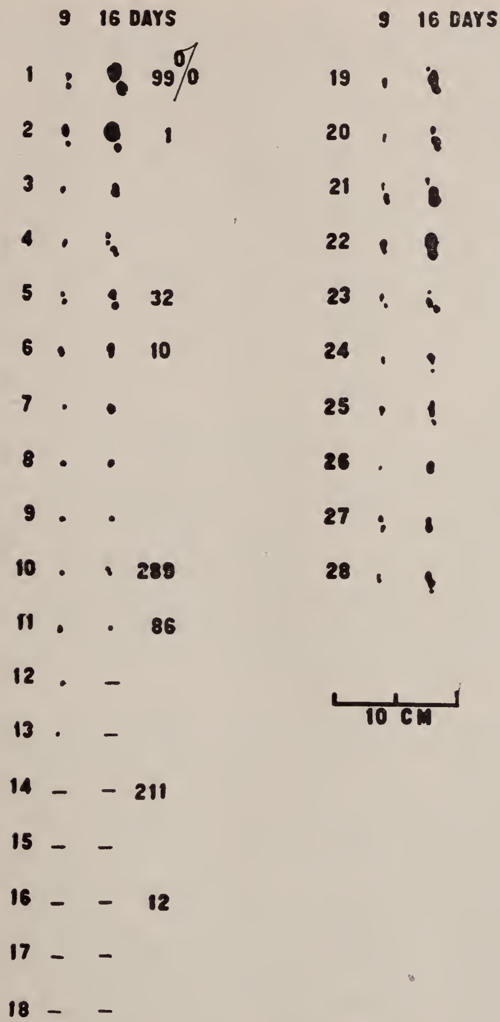


FIG. 2. EXPERIMENT $\frac{63}{128\,H}$

Mice 1 to 18 had their spleens measured March 14, 1916. Immunized March 29 with 0.05 cc. of emulsified spontaneous tumors. None of the inoculated material grew. Inoculated with 0.02 cc. of emulsified carcinoma 63 April 13. Killed and spleens measured May 2, forty-nine days after the first measurement (see table 5). The mice that proved immune did not all have enlarged spleens. Mice 19 to 28 are normal untreated controls.

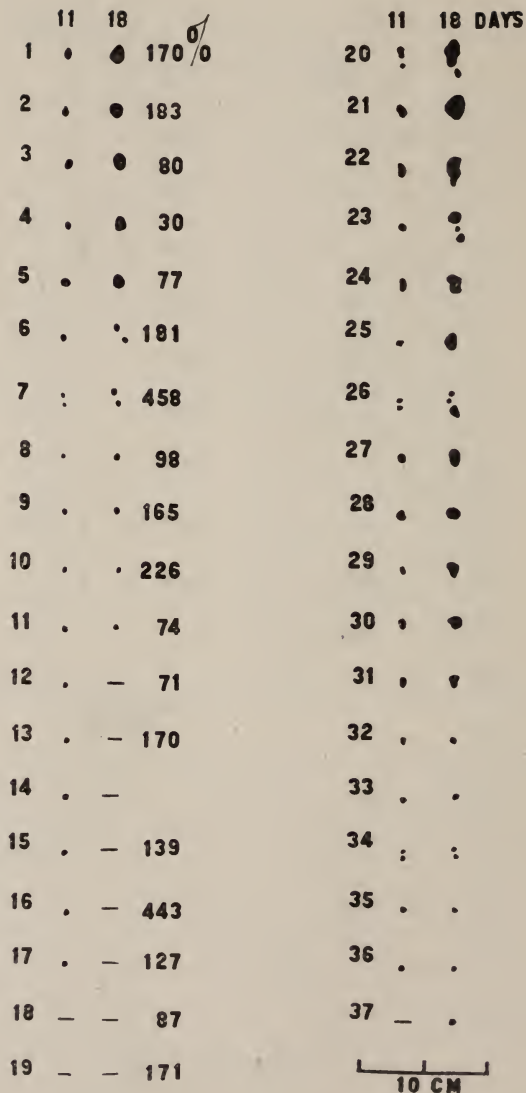


Fig. 3. Experiment $\frac{63}{130\text{ J}}$

Mice 1 to 19 had their spleens measured June 8, 1916. Injected June 15 with 0.05 cc. of emulsified spontaneous tumors. None of the inoculated material grew. Inoculated with 0.003 gm. of emulsified carcinoma 63 July 27. Killed and spleens measured August 18, seventy-one days after the first measurement (see table 6). Many of the spleens are enlarged, but the interval between the two measurements was long (see fig. 4). Mice 20 to 37 are normal untreated controls.

immunization and final measurement. On June 8, 1916, the spleens of thirty-six mice were measured (see table 6) and on June 15, seven days later, the animals were injected in the left axilla with a mixed emulsion of seven spontaneous tumors; no growths resulted from this preliminary treatment. Forty-two days after this (on July 27) the survivors (mice 1 to 19) were inoculated on the opposite side with 0.003 gram of carcinoma 63 by the needle method, and on August 18, sixty-four days following immunization, the survivors (Nos. 1 to 19) were killed and their spleens were measured. Mice 20 to 37 are normal untreated controls.

In this experiment an additional source of error has been eliminated, only mice with spleens less than 1.7 cm. in length having been employed (except that in No. 11 the spleen was 1.75 cm. long), so that this group contains only animals in which the organ approximated its normal size. The objection that in experiments 1 and 2 the spleen had already reached the limit of its ability to enlarge, and that an hypertrophy that might otherwise have taken place has thereby been masked, is here fully answered.

Eight of the treated mice (Nos. 12 to 19) were immune. In all but one of the eight (No. 14) the spleen had undergone an increase of more than 50 per cent of its original size; but it is to be remembered that these animals had been exposed to infection with mouse typhus for a longer period than those in the two experiments preceding. This, and not termination of the two preceding experiments before sufficient time had been given for the organ to enlarge, probably explains the high percentage of enlarged spleens in the immune mice of figure 3; for it will be immediately noted that among the whole group of nineteen mice, irrespective of whether or not tumors developed, only two mice escaped splenic hypertrophy.

The fact that enlargement of the spleen is a function of the length of time that a mouse has been kept in a cage with other mice, is well brought out by figure 4, which represents graphically the percentage of animals with enlarged spleens in the eight experiments discussed in this paper; the time is the number of days between the first and final measurement of the spleen.

The curve, fairly regular for a biological experiment, shows that irrespective of whether or not he is immune to propagable neoplasms, the longer a mouse is exposed to mouse typhoid the greater is the chance that his spleen will undergo hypertrophy. Thus 20 per cent of the mice kept together in a cage for fourteen days developed splenic hypertrophy, whereas from 77 to 89 per cent of those exposed for more than seventy days had enlarged spleens. Between these extremes the curve is comparatively uniform, except for a break at one point which cannot be explained. The most obvious way to account for it is to assume that this group was a healthier lot of mice; yet the number that died before the completion of the experiment was no less than in other experiments. The data from which the curve was drawn may be summarized as follows:

		TABLE	DAYS	PER CENT
No tumor	No preliminary treatment	1	14	20
$\frac{206}{177\ L}$ and $\frac{206}{178\ J}$	Immunized with tumor	9	39	39
No tumor	No preliminary treatment	2	47	47
$\frac{63}{128\ H}$	Immunized with tumor	5	49	22
$\frac{63}{128\ I}$	Immunized with embryo	4	61	50
$\frac{63}{130\ J}$	Immunized with tumor	6	71	89
$\frac{63}{134\ T}$	Injected with boiled embryo	8	78	77
$\frac{T}{90\ H}$	Injected with boiled tumor	7	126	77

The conclusion that the splenic enlargement in the immune mice of the preceding experiment is not due to their immunity is supported by the results of the next two experiments, in which the situation is analyzed still further. Boiled mouse tissue, which is incapable of producing resistance to transplantable tumors, was inoculated and the mice were set aside for a long period as before.

In experiment $\frac{T}{90\ H}$, figure 5, the spleens of thirty-six mice were measured on October 31, 1916, and all those with spleens

exceeding 1.7 cm. in length were rejected (table 7). Into the left axilla of these animals there was introduced, six days later, 0.1 cc. of a mixed emulsion of three spontaneous tumors that had been boiled for ten minutes. On January 3, 1917, sixty-four days following preliminary measurement, the thirty survivors were inoculated in the right axilla with 0.003 gram of carcinoma T by the needle method. The outcome of this inoculation (mice 1 to 30) was similar to that in untreated controls (mice 31 to 50) inoculated at the same time and with fragments of the same tumor.

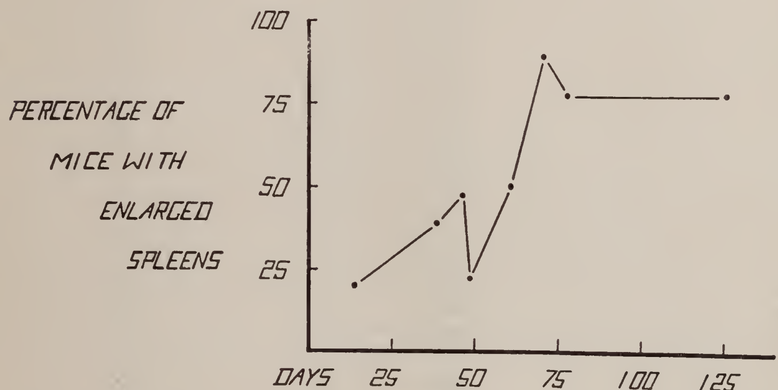


FIG. 4. The curve shows that the longer mice are exposed to mouse typhoid by being kept together in a cage, the greater is the chance that they will develop splenic hypertrophy.

Although the tumor grew in mice 1 to 8 fully as well as it did in the untreated controls, the spleen in all these eight mice was found enlarged more than 50 per cent of its original size at autopsy March 6, 1917. And the only mice in which there is less than 50 per cent increase in volume are to be found among Nos. 9 to 30, in which the tumor grew poorly, or not at all.

A similar experiment ($\frac{63}{134\ T}$) is reproduced in figure 6 (table 8). On October 30, 1916, the spleens of thirty-six mice were measured; the series includes only those animals in which this organ was 1.7 cm. or less in length. Two days later (November 1) the mice were injected in the right axilla with 0.1 cc. of an

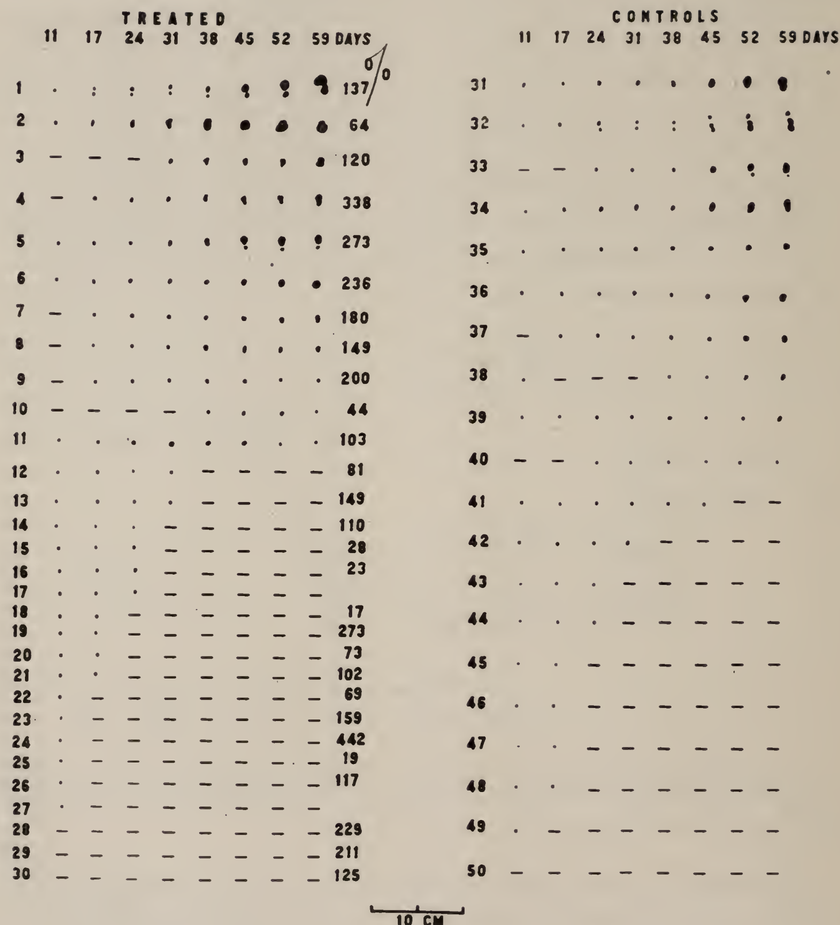


Fig. 5. Experiment $\frac{T}{90\,H}$

Mice 1 to 30 had their spleens measured October 31, 1916. Injected November 6 with 0.1 cc. of a spontaneous tumor emulsion that had been boiled for ten minutes. Inoculated January 3, 1917 with 0.003 gm. carcinoma T. Killed and spleens measured March 6, 1917, 126 days after the first measurement (see table 7). Many of the spleens are enlarged, but the interval between the two measurements was long (see fig. 4). Mice 31 to 50 are normal untreated controls.

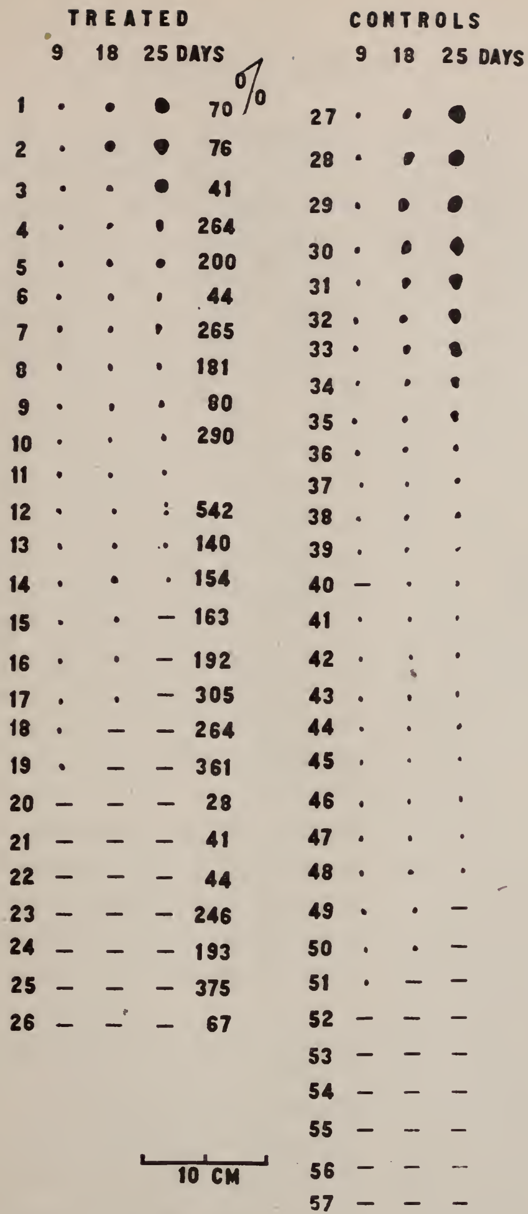


FIG. 6. EXPERIMENT $\frac{63}{134\,T}$

Mice 1 to 26 had their spleens measured October 30, 1916. Injected November 1 with 0.1 cc. of total embryo and placenta boiled for ten minutes. Inoculated December 21 with 0.002 gm. carcinoma 63. Killed and spleens measured January 16, 1917, seventy-eight days after the first measurement (see table 8). Although boiled tissues do not immunize, many of the spleens are enlarged. Mice 27–57 are normal untreated controls.

emulsion of total mouse embryo and placenta that had been boiled for ten minutes, and fifty days after this treatment the absence of immunity was demonstrated by inoculation into the right axilla of 0.002 gram of carcinoma 63 by the needle method. The mice were killed and their spleens were measured on January 16, 1917—seventy-six days after treatment with the boiled material. The outcome of the tumor inoculation is about the same in the surviving mice of the treated series (1 to 26) as it is in the normal controls (27 to 57). As in the controls, a number of the treated mice were more or less refractory, but there seems to be no relation between the resistance and the size of the spleen. The absence of more than 50 per cent increase in mouse 3, and the slight excess over this figure in mice 1 and 2, the three mice with the largest tumors, might appear at first glance to be suggestive, until it is noticed that in three of those that were entirely resistant (Nos. 20, 21, and 22) the spleen showed no enlargement above the personal error, and that in a fourth (No. 26) the increase was slight. In mouse 11, again, whose tumor remained stationary, the spleen was smaller than at the first measurement.

It cannot be objected against the two preceding experiments that the spleens which were enlarged had increased in size because tumors T and 63 during their proliferation had immunized the mice, for Russell (33) has shown that both these neoplasms belong to a type of new growth which does not immunize the host during its sojourn.

Since the animals had received no immunizing treatment, and since their tumors had not rendered them refractory, there is no explanation for their splenic hypertrophy except: (a) infection of one sort or another (probably with the bacillus of mouse typhoid); or (b) natural immunity. The latter alternative will be taken up presently, and shown to be inadequate.

In the meantime, it is necessary to meet a really valid objection which may be raised against the preceding experiments: That the resistance engendered by the introduction and absorption of living tissue, as in the three first experiments, is different from that evoked by a growing tumor, and that it may not be

accompanied by splenic hypertrophy. It is fortunate that the work of Russell previously referred to has given us another method of producing immunity at will. This author has proved that although certain transplantable neoplasms do not immunize the host during their residence therein, certain others will produce a highly efficient resistance, so that reinoculation with these or other strains will be unsuccessful. Such a neoplasm is carcinoma 206, which will immunize practically every mouse in which it has grown for about two weeks, as Russell has so well shown. This tumor was therefore employed as follows (experiment $\frac{206}{177\,L}$ and $\frac{206}{178\,J}$, fig. 7). The spleens of a group of thirty-six mice were measured (table 9) on March 23, 1916, and thirteen days later (April 5) these mice were inoculated in the right axilla with 0.02 cc. of an emulsion of carcinoma 206. In order to prove that they were immune, they were inoculated twelve days later in the opposite axilla with a similar dose of the same tumor; twelve normal untreated mice (Nos. 23 to 34) were inoculated at the same time and with the same emulsion as a control to the reinoculation. In figure 7, Nos. 1 to 22 are the surviving animals with measured spleens, and the tumors to the left of the black line are those resulting from the first inoculation, while the dashes to the right of the line show the outcome of reinoculation. All were immune. The controls, mice 23 to 34, show that the material used for the second inoculation was able to grow in nearly half of the twelve normal mice inoculated; hence it should have grown also in about the same proportion of mice 1 to 22, had these not been made refractory by the first implantation. It would be difficult to devise a more exact experiment than this in cancer research, for here one has twenty-two mice with spleens of known size, and definitely proved to be immune, for the negative results of the reinoculation were corroborated at autopsy (May 1, 1916). Yet in fourteen of the twenty-two (64 per cent) the spleen has not increased 50 per cent in volume; in one of these it was the same size, and in seven actually smaller.

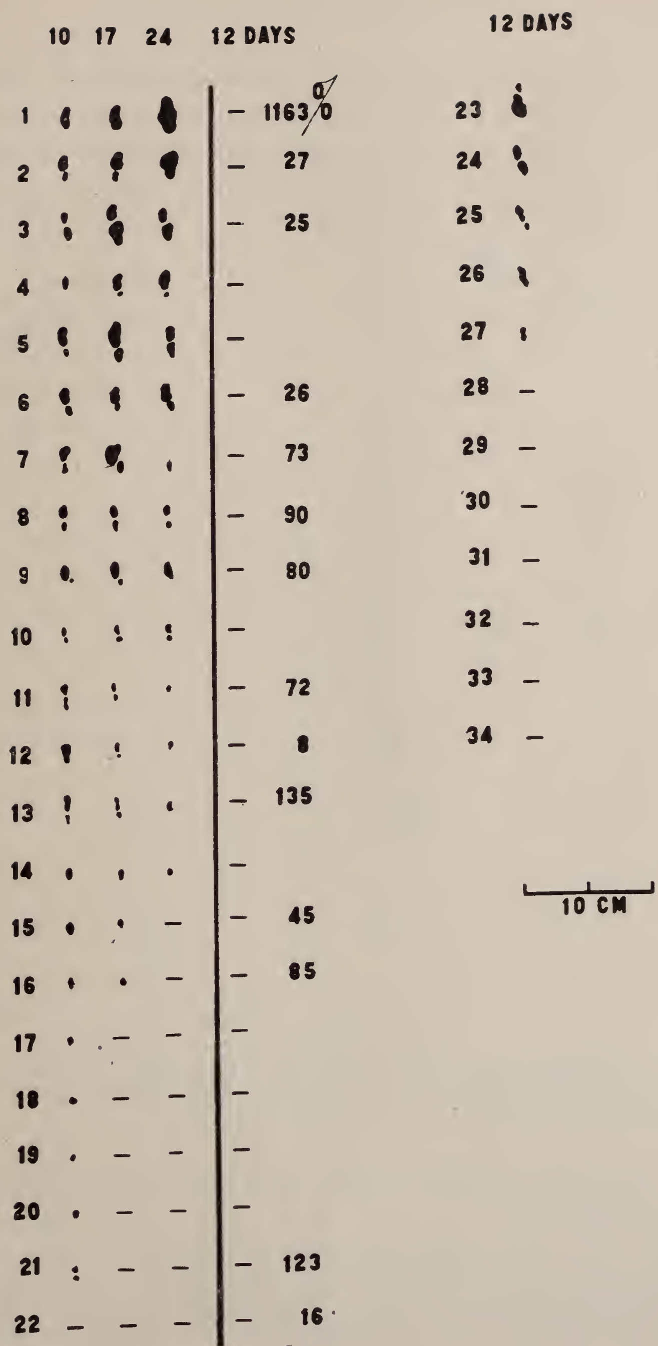
It was suggested in a preceding paragraph that certain mice may have had enlarged spleens because they were naturally

immune, though such an hypothesis loses much of its value when it is recollected that many mice with actively growing tumors have large spleens. And the assumption is still further undermined by the demonstration (figs. 8 and 9) that mice with splenic hypertrophy are not resistant to tumors. These figures reproduce the results following inoculation of two different mouse carcinomata into mice with enlarged spleens (tables 10 and 11). The outcome, on the left-hand side of each figure, is to be compared with growth of the same tumor, inoculated at the same time and in similar amount, into mice with spleens of approximately normal volume, on the right-hand side. Splenic hypertrophy evidently confers not the slightest resistance.

It is admitted, however, assuming for the moment that the refractory state does increase the volume of the spleen, that other causes might enlarge it as well; indeed, it has been proved that they actually do. Therefore, even though immunity does bring about hypertrophy, a mouse might have an enlarged spleen and still not be refractory to tumor inoculation, because the hypertrophy had been produced by some unrelated factor. But if immunity and splenic enlargement do go hand in hand, it may well be supposed that the mouse with the largest spleen will be most highly immune. Table 10 does not support this view. The two largest spleens (0.480 cc. and 0.441 cc.) belong to mice 8 and 10 (fig. 8), in which the tumor grew progressively. And while it is true that mice 1, 2, and 3, which developed large growths, had the smallest spleens in the series (0.170 cc., 0.170 cc., and 0.135 cc.), it is also true that mice 4, 5, and 9, with tumors

FIG. 7. EXPERIMENT $\frac{206}{177\,L}$ AND $\frac{206}{178\,J}$

Mice 1 to 22 had their spleens measured March 23, 1916. Immunized by the growth of carcinoma 206, 0.02 cc. of which was inoculated as an emulsion April 5. The results of this inoculation appear to the left of the vertical line. On April 17 the immunity of these mice was proved by inoculation of 0.02 cc. of an emulsion of the same tumor into the opposite axilla. The negative outcome of this inoculation twelve days later is shown to the right of the vertical line. The mice were killed and their spleens measured May 1, 39 days after the first measurement (see table 9). There is no relation between immunity and splenic hypertrophy. Mice 23 to 34 are normal untreated controls to the second inoculation.



10 17 24 12 DAYS
12 DAYS
1 — 1163/0
2 — 27
3 — 25
4 —
5 —
6 — 26
7 — 73
8 — 90
9 — 80
10 —
11 — 72
12 — 8
13 — 135
14 —
15 — — 45
16 — — 85
17 — — —
18 — — —
19 — — —
20 — — —
21 — — — 123
22 — — — — 16
23
24
25
26
27
28 —
29 —
30 —
31 —
32 —
33 —
34 —
10 CM

Fig. 7

almost equally large, had spleens (0.264 cc., 0.180 cc., and 0.216 cc.) no smaller than many of the immune animals. The average size of the spleens of the ten mice with progressively growing tumors is 0.245 cc., and of the ten immunes, 0.261 cc.; the latter exceeds the former by only 0.016 cc., or 6 per cent—a negligible

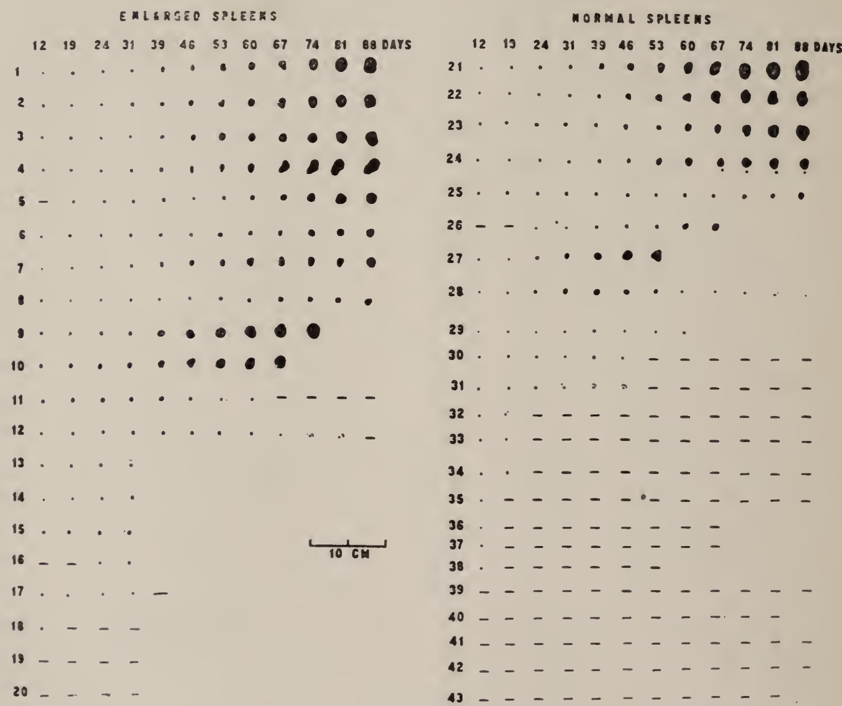


FIG. 8. EXPERIMENT $\frac{T}{89\ I}$

Mice 1 to 20 had enlarged spleens (see table 10). Mice 21 to 43 had spleens of approximately normal size (1.7 cm. or less in length). Mice with splenic hypertrophy are not resistant to tumor inoculation.

figure compared with the 50 per cent which has to be allowed for personal error.

Table 11 shows even more definitely the lack of any relation between immunity and the size of the spleen. In mice 1 to 12 (fig. 9) the tumors grew progressively and more or less rapidly.

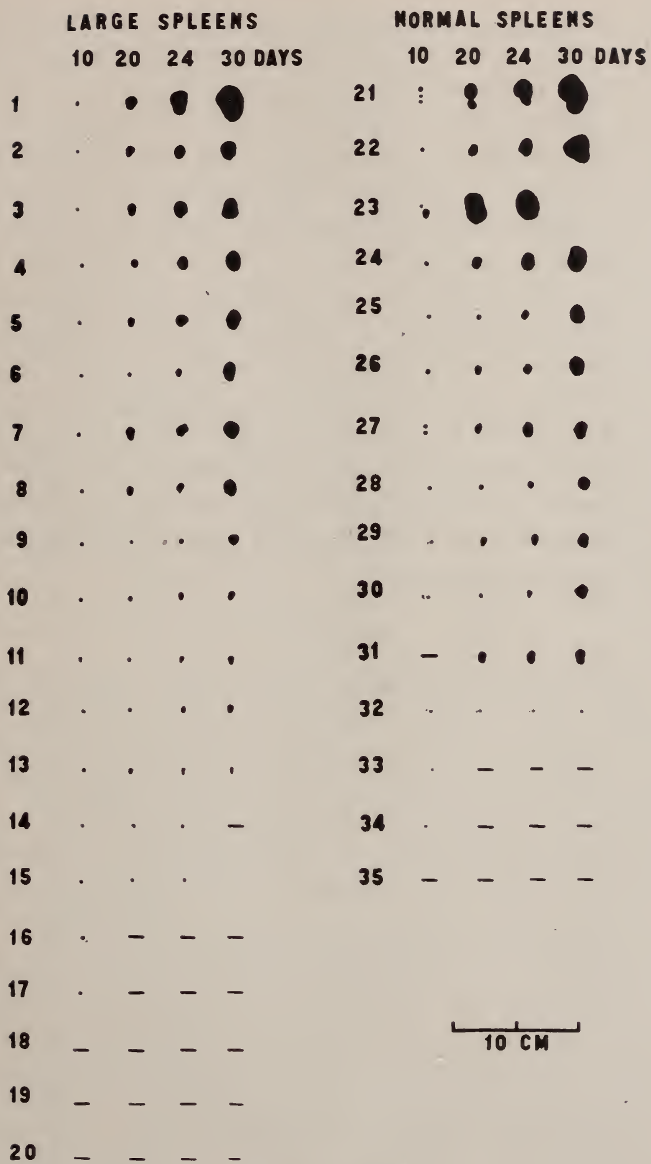


FIG. 9. EXPERIMENT $\frac{63}{134\ Q}$

Mice 1 to 20 had enlarged spleens and mice 21 to 35 spleens of approximately normal size (see table 11). Mice with splenic hypertrophy are not resistant to tumor inoculation.

Here again the two largest spleens (0.385 cc. and 0.374 cc.) are found in mice (Nos. 10 and 1) with progressively growing tumors. The two next largest (0.300 cc. and 0.286 cc.) are in resistant animals (mice 19 and 20), but the three next largest (0.273 cc., 0.252 cc., and 0.252 cc.) are in mice with progressively growing neoplasms (Nos. 2, 4, and 9). And though the three smallest spleens (0.127 cc., 0.140 cc., and 0.170 cc.) are found in mice with progressively growing tumors (Nos. 12, 11, and 7), three almost equally small (0.153 cc., 0.157 cc., and 0.157 cc.) occur among the immunes (Nos. 14, 16, and 18). The average size of the spleens in the mice with progressively growing tumors is 0.235 cc., while in the immunes it is somewhat less—0.211 cc. The difference, 0.024 cc. (or 11 per cent) is closely equivalent to that in the preceding experiment, except that the larger spleens are found now, not in the resistant, but in the susceptible mice. This difference, however, like the first, may be disregarded, being far below the personal error.

DISCUSSION

The two experiments taken together show that the presence or absence of an enlarged spleen in natural, as in acquired immunity, is a matter of pure chance so far as susceptibility or resistance to tumors is concerned. Splenic enlargement, therefore, is obviously the result of extraneous factors, and can often be traced to mouse typhoid, as has been proved in the first part of this paper. In a few instances, it is undoubtedly due to a condition resembling leukemia in the human subject.

The absence of any connection between immunity and splenic hypertrophy cannot, of course, be used to rebut the argument of such observers as Da Fano (34) and Murphy and his collaborators (35), who regard the lymphocyte as an important link in the process of immunization. For it is obvious that the spleen might be actively engaged in bringing about the refractory state, and yet show no evidence of its participation in so far as its size is concerned.

Although not quite germane to the subject discussed in the present paper, because they deal with spontaneous neoplasms,

the observations of Ewing (36) are nevertheless of interest in this connection. As the result of his own and others' experience at the autopsy table, this author denies any uniform relation between the size and condition of the spleen and the presence of cancer in the human body, and goes on to point out that cancer is, in general, a disease of elderly persons, in whom the lymphoid tissues and the spleen might reasonably be expected to show some physiological decline as compared with youthful subjects. On the other hand, actively growing tumors may be found in young patients with abundant lymphoid tissue. His conclusion that no information can be drawn from post mortem experience to favor the hypothesis that any part of immunity to spontaneous new growths is referable to the lymphatic apparatus, is shared by Wells (37), who has observed that spontaneous tumors frequently develop in mice with lymphatic or myelogenous leukemia; and that even where there is a prodigious increase in the number of lymphocytes, the growth is entirely unaffected.

These observations of Ewing and of Wells are not quite analagous to those discussed in the present paper because they deal with spontaneous new growths, and an animal cannot be immunized against his own tumor, as Haaland (38) has shown. An interesting experiment has been reported by Wood (39), who found that a transplanted tumor grew quite as well in a leukemic mouse as it did in a normal one. Still this case is not quite identical with those discussed in the experimental portion of this paper, because a mouse cannot be immunized with its own tissues (40, 41, 42).

This is, indeed, a fundamental weakness in Murphy's work—that animals so far have been immunized only with the tissues of others of the same species, never yet with their own. Therefore if the lymphatic system of a mouse is able to protect the animal against a new growth, it must be through the induction of some sort of immunity with which we have no acquaintance at the present time.

SUMMARY

While it is not denied that the spleen is concerned in bringing about immunity to propagable neoplasms, there is no evidence to show that the refractory state in mice is regularly accompanied by any enlargement of this organ appreciable to measurement. Some immune mice have enlarged spleens and some have not; some animals with progressively growing tumors have enlarged spleens and some have not. And the existence of other causes of splenic hypertrophy, such as mouse typhoid, transfers the burden of proof to those who assert that splenic hypertrophy is referable to immunity.

REFERENCES

(1) Cimoroni: Tumori, 1911–12, i, 691.
(2) Brancati: Tumori, 1911–12, i, 189.
(3) Price-Jones: Arch. Middlesex Hosp., 1911, xiii, 56.
(4) Mazza: Tumori, 1914, iv, 257.
(5) Price-Jones: Arch. Middlesex Hosp., 1913, xxx, 162.
(6) Apolant: Ztschr. f. Immunitätsforsch., 1913, Teil 1, Orig., xvii, 219.
(7) v. Hansemann: Deutsch. med. Wchnschr., 1915, xli, 633.
(8) Kettle: Jour. Path. and Bacteriol., 1912–13, xvii, 40.
(9) Graf: Centralbl. f. Path., 1910, xxi, 723.
(10) Goldmann: Beitr. z. klin. Chir., 1911, lxxii, 1.
(11) Endler: Ztschr. f. Krebsforsch., 1915, xv, 339.
(12) Brancati: Tumori, 1911–12, i, 189.
(13) Apolant: Ztschr. f. Immunitätsforsch., 1913, Teil 1, Orig., xvii, 219.
Brancati: Tumori, 1912–13, ii, 74. Cited by Apolant.
(14) Bullock and Rohdenburg: Jour. Cancer Research, 1917, ii, 465.
(15) Oser and Pribram: Ztschr. f. exper. Path. u. Therap., 1912–13, xii, 295.
(16) Brancati: Tumori, 1912–13, ii, 74.
(17) Rohdenburg, Bullock, and Johnston: Arch. Int. Med., 1911, vii, 491.
(18) Biach and Weltmann: Wien. klin. Wchnschr., 1913, xxvi, 1115.
(19) Morris: Jour. Cancer Research, 1917, ii, 498.
(20) Mottram and Russ: Proc. Roy. Soc., Series B, 1917, xc, 1.
(21) Bullock and Rohdenburg: Jour. Cancer Research, 1917, ii, 465.
(22) Braunstein: Berl. klin. Wchnschr., 1911, xlviii, 2029: Ztschr. f. Immunitätsforsch., 1913, Teil 1, Orig., xviii, 330.
Lewin and Meidner: Ztschr. f. Krebsforsch., 1912, xi, 364.
(23) Morris: Jour. Cancer Research, 1917, ii, 498.
(24) Mottram and Russ: Proc. Roy. Soc., Series B, 1917, xc, 1.
(25) Biach and Weltmann: Wien. klin. Wchnschr., 1913, xxvi, 1115.
(26) Da Fano: Ztschr. f. Immunitätsforsch., 1910, Teil 1, Orig., v, 1; Fifth Sci. Report, Imperial Cancer Research Fund, London, 1912, p. 57.

(27) Woglom: Ztschr. f. Immunitätsforsch., 1911, Teil 1, Orig., xi, 683.
(28) Memoirs of the Wistar Institute of Anatomy and Biology; No. 6; The Rat. Compiled and edited by H. H. Donaldson, Philadelphia, 1915.
(29) Medigreceanu: Proc. Roy. Soc., Series B, 1910, lxxxii, 286; Berl. klin. Wchnschr., 1910, xlvii, 588.
(30) Bashford: Tr. XVII Internat. Cong. Med., London, 1913. Sub-Section III (a), Chemical Pathology, p. 29.
(31) Rondoni: Ztschr. f. Immunitätsforsch., 1913, Teil 2, Ref., viii, 959.
(32) Loeffler: Centralbl. f. Bakteriol., 1892, xi, 129.
(33) Russell: Fifth Sci. Report, Imperial Cancer Research Fund, London, 1912, p. 1.
(34) Da Fano: Ztschr. f. Immunitätsforsch., 1910, Teil 1, Orig., v, 1; Fifth Sci. Report, Imperial Cancer Research Fund, London, 1912, p. 57.
(35) Murphy and Sturm: Jour. Exper. Med., 1919, xxix, 1 (bibl.).
(36) Ewing: Jour. Cancer Research, 1917, ii, 502; ibid., 1919, iv, 59.
(37) Wells: Jour. Cancer Research, 1919, iv, 57.
(38) Haaland: Fourth Sci. Report, Imperial Cancer Research Fund, London, 1911, p. 1.
(39) Wood: Jour. Cancer Research, 1919, iv, 57.
(40) Woglom: Ztschr. f. Immunitätsforsch., 1911, Teil 1, Orig., xi, 683.
(41) Apolant and Marks: ibid., 1911, x, 159.
(42) Lambert: Proc. Soc. Exper. Biol. and Med., 1911–12, ix, 18.

MULTIPLE TUMORS OF THE MOUSE MAMMA: ARE THEY INDEPENDENT OR METASTATIC?

ALBERT FISCHER

From Dr. F. G. Gades Pathological Institute, Bergen, Norway

Received for publication, May 15, 1919

Malignant neoplasms in human beings begin, as a rule, as single tumors, and the multiple growths which later appear are, as is well known, due to secondary spreading from this primary focus. Still multiple primary tumors are now and then to be met with; in the articles of Harbitz (1), Tanberg (2), de Besche (3), and Wolff (4), large series of such cases have been collected and discussed.

Harbitz arranges four groups of such multiple tumors:

1. Tumors of the same nature and in the same organic system.
2. Tumors of the same nature but in different organs.
3. Tumors of different nature and in different organs.
4. The carcinosarcoma.

Most of these tumors showing primary multiplicity are benign; the genuine malignant tumors, on the contrary, are less often primarily multiple in human beings.

To a certain degree the case appears to be otherwise with certain animal tumors, especially those in the mamma of mice, which are often apparently multiple. There are frequently found several independent growths in different parts of the mammary apparatus, which, in mice, is spread over a great deal of the body. Apolant (5) found in 221 cancerous mice about 12 per cent with multiple tumors; Murray (6), among 119 mice, about 15 per cent; and Haaland (7), in 288 mice, about 17 per cent. Multiple primary tumors appear to occur still more frequently in a strain of mice at Dr. F. G. Gades Pathological Institute (about 600 tumor mice), which will be described in a future publication from this laboratory. In almost all these cases the tumors were adeno-

carcinomata, appearing at widely separated points in the mammae, and thus belonging to Harbitz's group 1. In their multiplicity these tumors differ from those of human beings, and they have therefore been closely examined to see whether they actually are multiple primary tumors, or metastatic growths from a single primary focus.

When the various tumors show a completely different histological structure, there can be no doubt of their primary multiplicity. Often, however, they are similar and it is the latter case which will here be taken up, for the possibility that these are metastatic cannot be easily dismissed. To small histological differences much importance cannot be attached, as there are many cases where secondary tumors present considerable histological differences from the primary. Here it will be possible only in exceptional instances to produce proof that the growths are really multiple, and often one must be content with a probability. Where several rudimentary tumors appear at the same time and display about the same size, it is considered that one has a certain right to regard them as primary, and this is the case with the greater bulk of the cases which are reckoned as multiple tumors. Where there is a difference in the time of their appearance, or a considerable difference in their size, the matter will be much more difficult. But where the new tumors appear at widely separated points in the same organ system (mamma), and in the absence of metastases in other localities, especially in the interjacent tissues, it is more than probable that they are of wholly independent origin. For it is not likely that, if such tumors really are metastases, they should appear only in distant mammae, particularly as there does not appear to be any direct means of communication (Haaland (7)). If these cases can be included, the frequency of multiple tumors in mice will be considerably greater than is expressed by the figures previously mentioned; but one must exert his critical faculties to the utmost to exclude the possibility of metastasis.

Thus when all is considered, it appears probable that the multiple tumors of mice are independent, and not metastatic growths, though no direct proof has been produced. As the point is of

considerable interest, and our knowledge of the anatomical conditions upon which the question turns are defective, an attempt has been made to examine these conditions somewhat more closely. The method employed was the injection of insoluble particles into the mamma and a study of their transportation and deposition, as it may be assumed that tumor cells will in all likelihood follow the same path.

The technic was as follows: India ink was injected into the breasts through the nipple by means of a small syringe with a very fine needle. The quantity injected was about 0.1 cc. Female mice which had developed spontaneous tumors in one or more mammae were used for the experiments. In some of the animals the injection was made into one of the still normal breasts, in others into the tumor itself. The ink was introduced by preference into the inguinal mammae, which were found easier to inject.

The results were as follows (cf. table):—

1. When India ink was injected into the normal breast, it always appeared in the regional lymph-nodes. At first the periphery, later the entire node, was of a more or less intense black color.

2. In the next stage the course of the India ink could be followed to the central nodes and on to other lymph-nodes on the same side, and in individual cases ink has been observed in nodes on the opposite side.

3. No ink has ever been found in another mamma.

4. When India ink was injected into mammary tumors, the same occurrence was noted, except that transportation took place much more rapidly, both to the regional nodes and to those lying above and below them. Here, too, ink was never transported to other mammae.

The conclusion may be drawn that small solid particles, like those of India ink, travel from the mamma through the lymph-channels to the regional nodes, and then on to more distant lymph-nodes, as they do in the human subject. Direct transport from one breast to another, such as might permit multiple tumors to be regarded as of metastatic origin, has not been observed.

TABLE 1

NUMBER	SITE OF INJECTION		KILLED AFTER INJECTION	RESULTS			
	Mamma	Tumor		Regional nodes	Other nodes on same side	Nodes on opposite side	Other organs
			days				
512		R. ing.	1	+	Mediastinal and abdominal.	–	–
521	L. axil.	–	1	+	–	–	–
517	L. ing.		1	+	Abdominal.	–	–
524	L. ing.		2	+	Axil., abdominal.	–	–
527	L. ing.		2	+	Abdominal, axil.	Ing.	–
534		Back	2	–	–	–	–
461		L. vulvar.	2	+	Axil.	–	–
460	R. ing.		2	–	–	–	–
475	R. ing.		2	+	Axil., abdominal.	Axil.	–
485	L. ing.		3	+	Axil.	–	–
496	R. ing.		3	+	Abdominal, mediastinal.	–	–
542	R. axil.		3	+	Axil., cerv.	Axil.	–
531	L. ing.		3	–	Axil.	–	–
533	R. ing.		3	+	Mediastinal, axil.	Ing., axil.	–
526	R. axil.		4	+	–	–	–
525	L. axil.		4	+	–	–	–
536*	R. ing.		4	+	Axil.	–	–
532	L. ing.		5	+	Abdominal.	–	–
535		L. ing.	5	+	Mediastinal, axil.	–	–
540	L. ing.		5	+	Abdominal, axil.	–	–
543	R. ing.	L. ing.	5	++	–	–	–
490	L. ing.		5	+	–	Axil.	–
497	R. vulvar.		5	+	Mediastinal, axil.	–	–
483		L. ing.	11	+	Axil.	–	–
491	L. ing.		11	+	–	–	–
480		L. ing.	15	+	–	–	–
501	R. ing.		15	+	Abdominal, axil.	Axil.	–

* In this case the India ink wandered directly from the inguinal lymph-nodes through a lymph-channel in the skin up to the axillary nodes, without the central (abdominal) nodes being blackened. Here there was no black coloration of the interjacent breast-tissues.

Therefore the multiple new growths which appear so frequently in the mammae of mice are to be explained as independent, rather than as metastatic tumors.

(1) HARBITZ: Norsk Mag. f. Lægevidenskaben, 1916, p. 77.
(2) TANBERG: ibid., 1906, p. 983.
(3) DE BESCHE: ibid., 1908, p. 540.
(4) WOLFF: Die Lehre von der Krebskrankheiten, Jena, 1911, II Teil, 406.
(5) APOLANT: Cit. by Haaland.
(6) MURRAY: Cit. by Haaland.
(7) HAALAND: Fourth Sci. Report, Imperial Cancer Research Fund, London, 1911, p. 23.

ATTEMPTS TO OBTAIN A TRANSPLANTABLE TUMOR IN THE HIGHER SPECIES OF ANIMALS

F. C. MANN

Department of Experimental Surgery, Mayo Clinic, Rochester, Minnesota

Received for publication, March 24, 1919

Many attempts have been made by different investigators to transplant tumors in the higher species of animals, but all such experiments, with the exception of those dealing with the infectious sarcoma of the dog, have been failures. As the number of animals employed in any particular series has always been small, the chances of obtaining a successful growth have not been so good as if a larger series had been made. The advantage of obtaining a transplantable growth in such a species as the dog is quite obvious.

The purpose in the present article is to report a series of experiments in which attempts were made to transplant tumors of the dog and cat. It was thought that the chances of success would be greatly enhanced if a large series of animals was employed. The data obtained seemed to be of sufficient value to justify publication.

I. METHOD OF EXPERIMENTATION

After a preliminary investigation of the best method of transplanting tissue from one animal to another, a technic was devised as follows: Under anesthesia, usually local, and with the employment of careful sterile technic, the specimen was removed from the tumor and immediately cut with a very sharp razor into slices about 1 mm. thick. These slices were spread on a glass or a cork surface. A trocar having an internal diameter of about 2 mm. was loaded with a piece of tumor by pressing its sharpened distal end into the tissue against the underlying surface of

glass or cork. Each transplant was therefore as thick as the original slice and of the same diameter as the internal diameter of the trocar. The stilette of this trocar had a blunt end. The skin of the area chosen for transplantation was prepared in the usual manner by shaving and treating with iodin, and a second trocar with a sharp stilette was then plunged into the tissue to the desired point; this second trocar had an internal diameter of a size that would just freely admit the passage of the first. After withdrawal of the sharp stilette, the small trocar was plunged into the same position, and the stilette of this trocar was used to push the transplant well out into the tissues. After withdrawing both trocars the wound was treated with iodine. The transplant thus injected was disk-shaped and measured approximately 2 x 1 mm. In the employment of this technic it is possible, with the aid of three assistants, to make thirty to forty transplantations in the course of an hour, and rigidly to observe the rules of sterile technic; moreover, a better chance of obtaining a viable transplant will be insured.

Since we desired to make the investigation as extensive as possible, the site of transplantation varied. Usually one graft only was injected into an animal, but in some instances two were employed, often from different portions of the tumor.

II. THE RESULTS OF TRANSPLANTING A MAMMARY CARCINOMA IN THE DOG

The donor in this series of experiments, a well bred Scotch collie, was given to Dr. C. H. Mayo for the purpose of having this investigation made.

Protocol of the donor, dog C185. The animal was admitted to the laboratory on October 25, 1917. Though old, the animal was in excellent condition and weighed 18.6 kgm. Examination showed a large, hard, nodular tumor measuring 8 cm. in diameter, protruding about 2 cm. outward on the right side of the abdomen, and involving the third mammary gland on that side. Pus could be expressed from a central aperture, about 1 cm. in diameter, the end of a sinus which seemed to extend to the base of the tumor. The adjacent axillary lymph-

node on the same side as the tumor was enlarged and hard. On October 31 a specimen of the tumor was removed and examined microscopically; it proved to be carcinoma. On November 1, portions of the tumor were removed and injected into 54 other dogs. Although in the first 15 animals the trocars used were rather large, for all subsequent transplantations the method here described was employed. On November 8 the enlarged lymph-node was removed; it was found greatly enlarged, measuring approximately 3 x 1 cm., and was very firm, while at the lower pole there was a white nodule about 8 mm. in diameter which

FIG. 1. PHOTOGRAPH OF DOG C 185 SHOWING TUMOR OF MAMMARY GLAND FROM WHICH THE TRANSPLANTS WERE MADE

looked like a metastasis. Transplants of this nodule were injected into 19 dogs, and one injection was made into the first mammary gland in the right side of the donor. On November 9 portions were again removed from the primary growth and transplanted into 24 other dogs, after which an injection was made into the first mammary gland on the left side of the donor. On November 24 fragments from the primary growth were transplanted for a third time, into 34 other dogs, and into the second and fourth right and third and fourth left mammary glands of the donor.

The animal remained in excellent health until November 25, when she became quite sick, refused food, and lost weight and strength. The scleras and mucous membranes became distinctly tinged with yellow. On December 2 the animal was bled to death under ether anesthesia, and necropsy (636–17) was performed immediately. The original growth was comparatively small. The only transplants to be found were in the fourth breast on the right side, and the third and fourth on the left side; at each of these three sites, areas were found in which there appeared to be viable transplants. The superficial and

Fig. 2. Photomicrograph of Original Tumor in Dog C 185 (× 125)

deep lymph-nodes on the left side were slightly enlarged, but did not appear to contain carcinoma. The thyroid had undergone cystic degeneration, and very little normal thyroid tissue remained. No thymic tissue could be found. The heart was flabby but otherwise appeared normal. The lungs exhibited many metastases ranging from 2.5 cm. to 2 mm. in diameter, every lobe containing one or more. In the lower part of the anterior mediastinum was a large irregular mass, which measured approximately from 10 to 12 cm. in diameter and was composed wholly of carcinoma. The duodenum and pancreas were adher-

ent to the peritoneal wall opposite the site of the original tumor. The gastro-intestinal tract was practically empty and normal, except for a few small superficial ulcers. The pancreas was studded throughout with small areas which appeared somewhat like fat necrosis but which, owing to their similarities and discreteness, were considered to be metastases; their size varied from 1 to 4 mm. in diameter. The liver was also studded with nodules, apparently metastatic, varying in size from 1 to 2 mm. to 2 cm. in diameter. The spleen and the right kidney appeared normal, but the left kidney contained a large metastatic

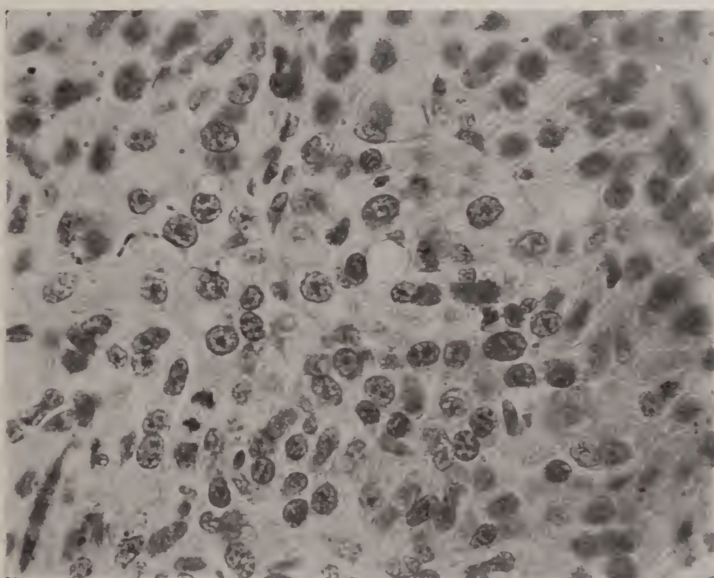


Fig. 3. Photomicrograph of Original Tumor in Dog C 185 (× 500)

nodule in the pelvis, measuring about 1 cm. in diameter, and two small ones in the cortex, each about 3 mm. in diameter. The left adrenal also showed a small metastasis. No other pathologic findings were noted.

The specimens were fixed in formalin and neutral formalin and Zenker. Histologically the primary growth was a typical mammary carcinoma. Metastatic carcinoma cells were found in the axillary lymph-node from which transplants were made, in the lungs, mediastinal lymph-nodes, liver, pancreas, kidney, and adrenal. The three transplants which at autopsy appeared to be viable did contain viable carcinoma cells.

Conditions and results of the injection experiments

Since it was our purpose to attempt to produce a transplantable tumor, as many animals as possible were injected. No attempt was made to study the immediate fate of the transplant, and end results only were observed.

1. *Number of injections.* The total number of dogs injected was 134; five of the animals were inoculated twice, so that the total number was 139, not including those made into the donor. One hundred and twenty were of the primary growth and 19 of the axillary lymph-node metastasis.

2. *Infections.* Owing to the fact that the primary growth was infected at the time the animal entered the laboratory, great difficulty was encountered in getting sterile specimens for transplantation. Infection, however, did not prove to be a very serious factor; there were ten definite infections, some of them severe, at the site of implantation, and eighteen slight infections. That these latter were not of great importance is proved by the fact that viable carcinoma cells were noted in some of the cases classified as "slightly infected." In 111 cases the healing was primary.

3. *Site of injection.* One hundred and eight of the injections were made subcutaneously in the left axillary region, which was chosen most often because it is easy to inject and to observe. The mammary gland seemed to be another favorable site, and 15 injections were made there, though of course in some instances the transplant did not penetrate into the substance of the gland. In puppies it was easier to inoculate into the groin, and 16 injections were made in that area. Three injections were made in the middle of the left nipple line, and one each into the testicle, into the peritoneal cavity, and into a granulating wound.

4. *Age of the animals injected.* The larger number of the dogs were young adults, although practically all ages were represented; the youngest were puppies only a few weeks old; a few were very old, particularly one, in whose mouth only six teeth remained.

5. Condition of the animals injected. Most of the dogs were in excellent condition, though a few were in very poor health, either because they had but recently been received in the laboratory, or because they were suffering from infection. Two or three of the animals had distemper at the time of injection.

6. Breed of the animals injected. A majority of the animals were mongrels with a preponderance of the characteristics of one particular breed. Several of them, however, were well bred. As the donor was a collie, it seemed desirable to employ as many collies as possible; four of the twelve such animals injected were pedigreed. Several of the animals of other breeds were known to be of pure stock.

7. Operative procedures to which the animals were subjected before injection. Approximately half of the animals employed in the series had been previously subjected to operative procedures. In most instances the operations could not have had any relation to a positive or negative result in the transplantation of the tumor, though in some cases the operation was regarded as a possible complicating factor. For example, in a few animals one ovary had been removed, one animal had been castrated before he was received at the laboratory, one had received many exposures to the *x*-ray, five had been splenectomized before, and four after inoculation.

8. The agglutinins in the recipients' blood. It was thought that possibly the different animals could be grouped like human beings with regard to agglutination of the blood, and that this grouping might have something to do with the growth of the transplant. Accordingly the corpuscles of 65 dogs were tested with the serum of the donor. Agglutination occurred in two instances only, which probably indicates that dogs cannot be grouped.

9. Results of transplantation. After injection the animals were closely observed. Some of them died from various causes, and others were used in subsequent experiments; thus most of them came to necropsy although several are alive one year after injection. In only six animals was the transplant found and in two of these it was definitely dead and necrotic. In four instances visible cancer cells were found at necropsy.

Protocols of experiments in which viable carcinoma cells were found at necropsy

1. Dog C29, a small, black, male terrier, weighing 7.6 kgm. was admitted to the laboratory in June, 1917. On June 22 Dr. Harrington sectioned and anastomosed the right ureter (experiment 453–17). The animal did very well immediately after the operation, but developed later a superficial abscess at the site of some non-absorbable suture material, gradually lost weight; in October the abscess was

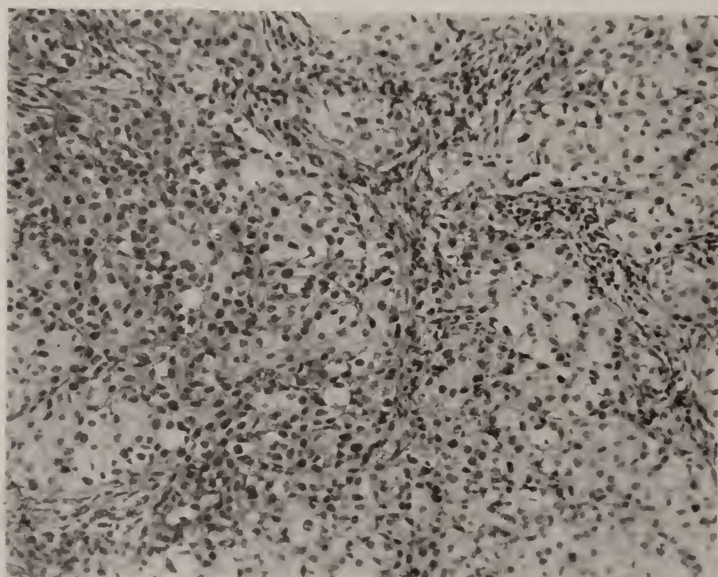


Fig. 4. Photomicrograph of Transplant of Dog C 28
Compare with original figure 2 (× 125)

opened and the foreign material removed. On November 1 two pieces of carcinoma, measuring approximately 2 by 2 by 1 mm. were removed from the donor (dog C185) and injected into the left axilla within less than one hour. The trocar wound closed, but on November 4 it seemed to be infected and a slight amount of seropurulent fluid escaped; there was, however, very little reaction around the site of injection. On November 9 the wound was healed, and the transplant could not be found. A small palpable nodule developed at the site of the transplant, but it could not be differentiated from a

lymph-node. The animal remained in poor condition and died suddenly on December 11, forty-one days after the injection of the carcinoma specimen.

This experiment came the nearest to success. At necropsy (612–17), which was performed shortly after death, a nodule could be palpated at the site of the injection. On dissection this was found to be a small discrete mass, light in color and appearing very much like the primary carcinoma of the donor. It was roughly elliptical in shape, measured

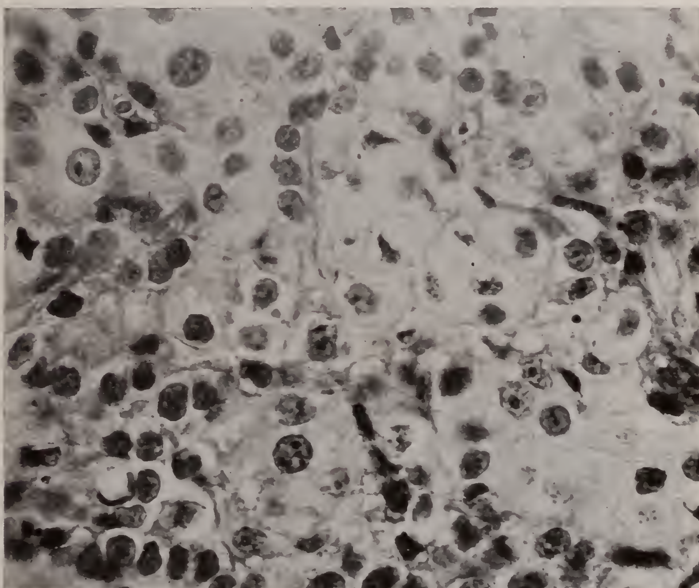


FIG. 5. PHOTOMICROGRAPH OF TRANSPLANT OF DOG C 28

Compare with original figure 3

4.3 by 3 by 3 mm., and had a capsule of its own and a well developed blood supply. It was very easily exposed and freed from surrounding tissue, and appeared to be the transplant.

The tissue was fixed in formalin and paraffin serial sections were made. Microscopically the tissue was shown to be the transplant, completely surrounded by a definite capsule. The carcinoma cells in many areas were situated in small groups, which were surrounded by well developed connective tissue, though there were small areas where the carcinoma cells were packed close together with very little intervening

connective tissue. In many areas there was distinct round cell infiltration, often following the bands of the connective tissue and thus penetrating between the groups of carcinoma cells. The carcinoma cells, especially in the areas which were infiltrated with round cells, were vacuolated and fragmentary. There were many large areas, particularly those in which the cells were packed closely together and in which the carcinoma cells were perfectly normal in appearance. In these areas it was possible to find a few carcinoma cells undergoing mitotic cell division. In this experiment there is no question but that the transplant lived and grew. In the forty-one days it remained in the new host it had more than quadrupled its volume, although it was walled off by a definite capsule. There was well marked evidence of beginning absorption, shown by the round cell infiltration and the vacuolization of the cells. On the other hand, many of the carcinoma cells were in excellent condition and a few mitotic figures showed that growth was still taking place. Whether the transplant would have grown much larger if the animal had lived longer is questionable; in all probability it would gradually have been absorbed. The other three experiments in which a viable transplant was found at necropsy will be briefly described:

2. Dog C240 died twelve days after the injection. The transplant was found to be undergoing necrosis and absorption; only a few viable carcinoma cells were present.

3. Dog C252 died twelve days after the injection. The transplant was undergoing necrosis and absorption, and only a few viable carcinoma cells were present.

4. Dog B981 died seven days after the injection. Most of the cells of the transplant were becoming necrotic although there were undoubtedly many viable carcinoma cells near the edge of the transplant.

It may be noted that in each of these three experiments the length of time between the injection of the transplant and the death of the animal was short.

III. THE RESULTS OF TRANSPLANTING A FIBROMA IN THE CAT

Protocol of the donor, cat 113. This animal, a yellow and white male weighing 2452 grams was admitted to the laboratory on December 22, 1917, in rather poor condition. In August, 1917, the owner had noticed

a small nodule on the external surface of the left ankle. This nodule grew and soon ulcerated, and at the time of examination measured about 2 by 2 cm., the whole external surface was ulcerated, the growth

Fig. 6. Photograph of Cat 113 Showing Tumor on Leg

Note the small nodule above tumor; this is the first transplant

Fig. 7. Photograph of Viable Axillary Transplant in Cat 113

The two white masses embedded in muscle are the transplants (natural size)

appeared to be firmly attached to the deeper structures and the infection extended deep into its substance. A small piece was removed for histologic study. On December 26 several pieces of the tumor were

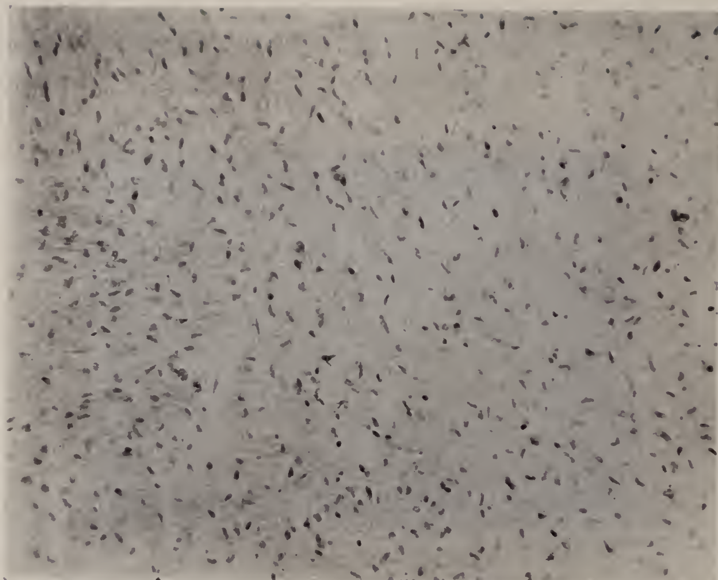


Fig. 8. Photomicrograph of Original Tumor in Cat 113 (× 125)

Fig. 9. Photomicrograph of Original Tumor in Cat 113 (× 500)

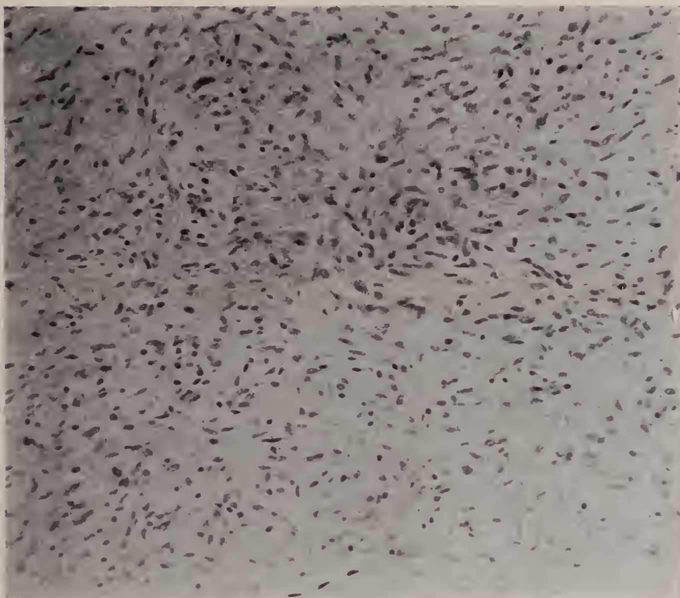


FIG. 10. PHOTOMICROGRAPH OF AUTOTRANSPLANT IN LEG OF CAT 113 (× 125)

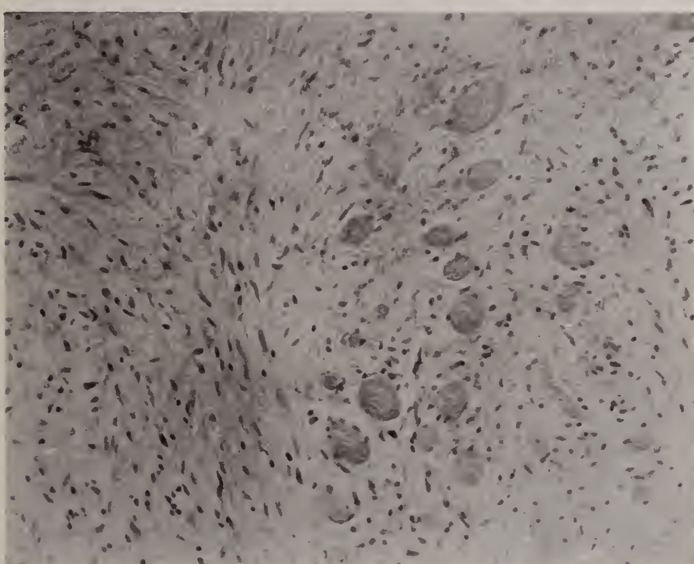


FIG. 11. PHOTOMICROGRAPH OF AUTOTRANSPLANT IN AXILLA OF CAT 113
Note bundle of muscle in the growing transplant

removed and transplanted into eight other cats, though it was practically impossible to obtain sterile pieces of the tumor. One transplant was made subcutaneously in the donor just above the original growth. Subsequently the ulcerated surface of the tumor was treated and all but the superficial infection was cleared up. The tumor continued to grow, and on January 29, 1918, it measured 4.5 by 3 by 3 cm. At this time sterile pieces could be obtained, and transplantations were made into seven cats, after which two transplants (two pieces) were injected

Fig. 12. Photomicrograph of Transplant in Cat 143, 76 Days After Transplantation

Compare with original figure 8 (× 125)

into the donor (cat 113), one into each axilla. The trocar wound on the left side, which became infected, necessitated drainage.

The animal remained in good condition, the tumor grew, and the ulceration under treatment remained localized. On February 11, specimens were transplanted into five cats. The transplants injected into the axillae of the donor could not be palpated, and after the five cats had been injected, two more transplantations were accordingly made into the right axilla of the donor. The transplant that had been

injected into the leg of the donor on December 26 could be palpated. The primary tumor grew slowly and the ulcerated surface healed, but the general condition was not good; the animal lost strength and weight and became anemic. On June 10 fragments were removed and injected into four cats. The donor was then etherized and bled to death.

Necropsy (289–18) was performed immediately after death. The original tumor extended rather deeply, but was not connected with the bone; it was encapsulated, though it could be dissected free

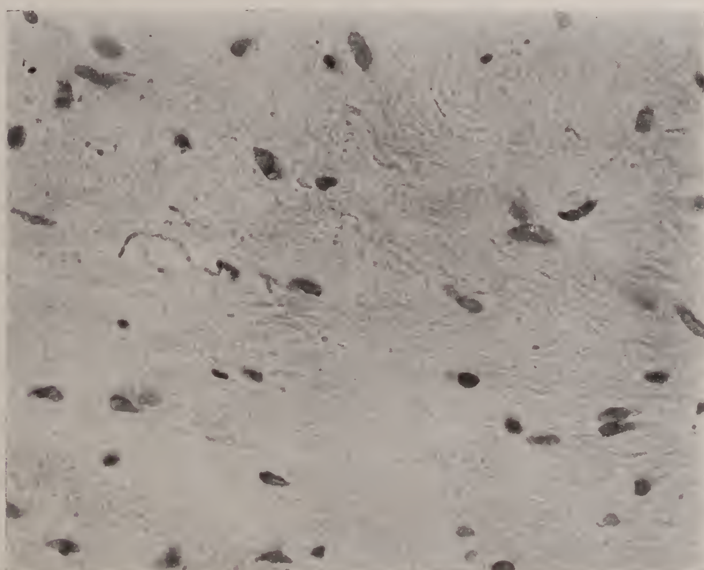


FIG. 13. PHOTOMICROGRAPH SHOWING A HIGHER POWER OF THE SAME SECTION AS FIGURE 12

Compare with figure 9 (× 500)

from the surrounding tissue only with difficulty. No metastases could be found; the lymph-nodes near the tumor were enlarged, but this was due to the infection. All the organs appeared normal. The first transplant, injected subcutaneously above the original tumor on the same leg, had grown, and measured 8 mm. in diameter; it was discrete and appeared similar to the primary growth. Two small nodules could be palpated in the left axilla. These when exposed were found buried in muscle and had the same appearance as the mother tumor;

they measured 7 by 6 by 6 mm. and 6 by 5 by 5 mm., respectively. They were undoubtedly the transplants that had been injected January 29. The transplants in the right axilla were found, but they were quite small, although they appeared viable.

The specimen that was removed from the tumor on December 22 was taken at a point close to the infected surface. Histologically it appeared to be a sarcoma. However, examination of specimens taken at a point somewhat removed from the ulcerated surface, and of all specimens removed after treatment, showed the growth to be a fibroma. The transplants appeared similar, histologically, to the mother tumor.

Conditions and results of the experiments

1. Number of injections. The total number of cats injected with transplants from the original tumor was 25. One animal was injected at two different times. The number of animals injected with a transplant from previous transplants was 7; total number 32. Many of the animals had more than one transplant injected; the total number of inoculations was 46.

2. Infection. When the donor was received at the laboratory the tumor was infected, which made it practically impossible to obtain sterile specimens. For that reason the first series of transplants had a very high incidence of infection. After the ulcerated surface had been treated and sterile specimens could be obtained, infection did not occur. Of a total of 46 injections only 6 were infected. It should again be noted that infection does not greatly decrease the chances of a viable transplant, for one of the trocar wounds in the donor (cat 113) became so badly infected that it required free drainage, yet the transplant grew.

3. Site of injection. The majority of the inoculations were made subcutaneously in the axillary region, over the xyphoid process, or near the umbilicus. A large number, however, were made into the peritoneal cavity.

4. Age. The youngest animal injected was about two thirds grown. A few of the animals were definitely aged, but most of them were young adults.

5. Condition. With one or two exceptions, the general condition of all the cats was excellent. None of the animals had been subjected to any previous operative procedure.

6. Breed. All the animals were mongrels.

7. Results. The total number of transplantations from the original tumor was 31. The transplant was found to have grown or remained viable in 12 cases. Two of these that appeared to be viable had been transferred for too short a time to be of any value in drawing conclusions. The other ten were found viable 9, 13, 18, 23, 25, 38, 46, 50, and 76 days after injection. All of these without doubt had grown in the new host. However, all that had remained long enough in the recipient had been gradually absorbed and there is no doubt that all would ultimately have been.

Transplants taken from a previous transplant were injected in 15 instances. Microscopically these transplants were found to contain at least a few viable tumor cells at the time of the second injection although in almost every instance death and absorption of the transplant were in progress. None of these sub-transplants remained viable.

8. Results of reimplantation of the tumor in the donor. After each series of transplants had been injected, one or two injections were made in the donor; thus five transplants were injected at three different times. Four of these grew. Only two, however, developed into tumors which were exactly similar in appearance, both grossly and histologically, to the mother tumor.

SUMMARY

The results are given of a series of experiments in which transplants of a mammary carcinoma of a dog were injected into 134 dogs, and of a series of experiments in which transplants of a fibroma of a cat were injected into 32 cats. A transplantable tumor was not obtained in either series of experiments, although a few transplants grew for a short time. The transplants of the fibroma which were made in the donor grew. The results obtained in these experiments, in which tumor was employed, are strikingly similar to the results of auto- and homo-transplantation of normal tissue. The problem of developing a transplantable tumor in the higher species of animals is, it would seem, closely allied to the problem of making homo-transplants of normal tissue grow.

A STUDY OF THE CHEMICAL COMPOSITION OF THE BLOOD IN CANCER

RUTH C. THEIS AND WILLIAM S. STONE

From the Huntington Fund for Cancer Research, Memorial Hospital, and the Harriman Research Laboratory, Roosevelt Hospital, New York

Received for publication, June 4, 1919

Since the micro-methods of chemical analysis have been developed, much work has been done on the chemical analysis of the blood in various pathological conditions, particularly in nephritis. Almost no work, however, has been done on the chemical analysis of the blood of patients suffering from cancer, although definite changes might be expected in a disease so marked by anemia and cell proliferation.

The sugar content of the blood in cancer has been a subject of discussion for many years. In 1885, Freund (1) regularly noted an increase, the figure being sometimes up to 0.33 per cent, which was confirmed by Trinkler (2). Matrai (3) maintained that such an increase was not characteristic of cancer but of the anemia which is secondary to the disease. Lewis and Benedict (4) using their new method, reported that 19 out of 53 cases of malignant disease showed a blood sugar figure above 0.11 per cent.

The alkalinity of the serum in malignant disease and other pathological conditions has recently been investigated by Maud Menten (5). In a series of 73 cases of malignant disease an increased alkalinity was noted in all except three or four. It was more pronounced in cancer of the internal organs than in superficial lesions. During pregnancy in which physiological cell-proliferation takes place, she found normal alkalinity. The increased alkalinity in cancer, however, is not specific for the disease, for it is found to be abnormally high in various skin diseases, and in diabetes mellitus in the stage preceding acidosis.

That the cholesterol content of the blood of cancer patients

is increased was reported by G. Luden (6), using the methods of Autenrieth and Funk (7) and Bloor (8). Considering the upper limit of cholesterol in normal blood to be 0.27 per cent, it was found in nine cases of cancer to vary from 0.26 per cent to 0.71 per cent, the lower values being found in cases that had been treated with radium. Denis (9), however, using the modified method of Bloor, reported a normal content in all but fourteen cases of early cancer, with the exception of one, in which a low content was associated with anemia.

We have determined sugar and the non-protein nitrogen constituents except creatinine and creatine in blood obtained from 189 patients in the wards of the Memorial Hospital, all of whom were suffering from malignant or allied disease such as leukemia or Hodgkin's disease. Many of the severe types of cancer were represented, and the majority of the cases were well advanced, so that changes in the blood should have been found if such changes are characteristic of the disease. The writers are indebted to the house staff of the hospital for their prompt and regular withdrawal of the blood. This was obtained from a vein in the arm each morning between 11.00 and 12.00, and defibrinated. As many of the constituents of the blood were determined on each specimen as the amount of blood withdrawn allowed. Duplicates were always done on non-protein nitrogen, urea, and sugar, and whenever possible on uric acid and amino acid nitrogen.

The methods used were:

Non-protein nitrogen: Greenwald's (10) modification of the Folin-Denis method. Distillation, according to Bock and Benedict (11) instead of aëration and direct titration with 0.01 N NaOH.

Urea: Marshall (12).

Uric acid: Benedict's (13) modification of the Folin-Denis method using the modified uric acid reagent (14).

Amino acid nitrogen: Bock (15).

Sugar: Lewis and Benedict (16).

For comparison of the figures obtained in the present study, it is necessary to consider briefly the normal value for the non-protein constituents of the blood.

As the result of the work carried out at Cornell Medical College and in certain other laboratories where the same methods have been used which we employed, we adopted the following figures as representative of normal values. Non-protein nitrogen, 28 to 35 mgm. per 100 cc. Urea nitrogen, almost exactly 50 per cent of the non-protein nitrogen, viz., 14 to 18 mgm. per 100 cc. Uric acid ranges between 1 and 2.5 mgm. per 100 cc.; amino acid nitrogen is about 7 to 8 mgm. per 100 cc., and sugar 90 to 110 mgm. per 100 cc.

In table 1 are presented the results of analyses of the blood from 189 cancer patients. The cases studied include a very wide range as regards type and malignancy of the disease. It is obvious that for any proper interpretation of the data presented account must be taken of the age of the patient, as well as of his general condition and the tissue involved in the disease process. Thus in cancer of the liver it would be quite unjustifiable to credit changes which might be found in the blood to the cancer process as such—these changes might be wholly due to disturbed metabolism resulting from improper functioning of the liver. Such objections would not apply to growths located in the breast or uterus, or to the various cases of purely superficial growths. Alteration of the blood constituents in such cases can properly be attributed to the cancer process, providing that these changes are apparent in a good percentage of the cases. The clinical data presented in table 1 have been condensed as much as possible, but it is hoped that enough of such data is included to enable one to judge whether the blood changes found can be ascribed to the disease, or must be referred to the impairment of some particular organ. The cases are classified in general groups according to the location of the disease.

An inspection of table 1 shows that the values found for non-protein nitrogen and urea are definitely below the normal. This is especially marked in the mammary and uterine cases, many of which show figures which are probably the lowest so far recorded for non-protein nitrogen and urea in human blood. Carcinomas of the breast and uterus are commonly recognized as representing the most malignant and rapidly growing tumors,

TABLE 1

NUMBER	AGE	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	AMINO ACID NITROGEN	SUGAR	REMARKS
				Uterus			
		mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent*	
1	40	28.0				0.088	Advanced early recurrence
2	31	26.6				0.073	Rapid. Terminal stage
3	55	30.8	14.7			0.110	Adenocarcinoma ovary. Recurrent; long duration
4	45	30.8	14.7			0.112	Far advanced acanthoma. Pyelitis
5	52	32.2	12.6			0.104	Localized. Percy. Radium
6	35	28.0	13.6			0.088	Slowly growing recurrence
7	44	28.7				0.088	Well advanced. Rapidly growing
8	49	25.2	14.0	1.2		0.111	Advanced. Short duration. Very malignant
9	53	32.9	15.4	3.3		0.100	Ovary. Advanced, but local
10	50	37.9	22.0			0.111	Superficial. Vulva
11	63	30.1	14.0	2.6		0.121	Long duration. Advanced
12	53	32.2	12.6	2.4	6.7	0.119	Advanced, but local
13	46	26.6	13.6	4.4	7.6	0.103	Short duration. Local. Immunity
14	44	32.2	15.7	1.2	9.3	0.101	Duration 1 year. Advanced, but not very malignant
15	65	31.5	13.3	2.2	7.7	0.107	Local and limited adenoma
16	44	33.6	12.6		7.1	0.100	Early lesion, plus syphilis. Cured
17	37	25.2	10.1	1.9	7.2	0.102	Well advanced
18	44	30.8	12.6	4.3	7.5	0.117	Immunity. Recurrent. Extensive. Slow growing
19	42	28.0	16.1	3.3		0.089	Well advanced. Adenocarcinoma ovary
20	40	35.1	17.5		7.6	0.090	
21	45	28.0	10.5	5.3	7.1	0.145	Far advanced
22	48	30.8	12.9	4.0		0.153	Well advanced. Nephritis
23	47	21.6	9.1	2.9	7.1	0.107	Advanced
24	58	24.5	12.9		6.5	0.097	Far advanced. Long duration. General carcinosis
25	53	36.5	18.2		6.9	0.101	Rapid. Very malignant. Advanced

TABLE 1—*Continued*

NUMBER	AGE	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	AMINO ACID NITROGEN	SUGAR	REMARKS
				Uterus.—Continued			
		mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent*	
26	55	37.9	23.5		9.8	0.135	Very malignant. Extensive recurrence
27	35	22.4	10.1		8.3	0.089	Very malignant
28	34	25.9	17.5			0.091	Recurrence. Advanced. Immunity
29	50	26.6	13.3		7.7	0.128	Very malignant. Kidneys
30	67	27.3	13.3			0.136	Local. Vulva. Degenerative changes of age
31	29	23.8	9.1		7.6	0.166	Advanced. Duration 1½ years. Hemorrhage
32	26	23.8	10.1		6.7	0.121	Very malignant. Rapid. Postoperative
33	32	25.2	10.8		7.6	0.106	Rapid. Very malignant
34	39	18.2		3.6			Short duration. Very malignant. Metastases
35	48	32.9		2.6			Metastases 5 weeks after operation
36	38	16.8		<1			Early. Local; cured
37	53	30.8	18.2	1.4	10.5		Bad general condition. Recurrence 3 years after operation
38	56	25.2	18.2		6.5		Advanced. Terminal stage
39	57	22.4		<1			Well advanced adenoacanthoma
40	48	22.4	5.6			0.107	Well advanced
				Breast			
41	50	22.4				0.122	Rapidly growing. Late stage. Male
42	43	30.8	16.1	2.2		0.113	Recurrent; advanced
43	34	28.7	12.9	3.8	13.4	0.091	Anaplastic; rapid; pregnant. Very responsive to x-ray
44	27	24.5	10.5		8.1	0.105	Anaplastic; pregnant. Rapid. Responsive to radium for short period
45	38	23.8	12.6		7.3	0.100	Recurrent. Advanced
46	68	34.3	14.7	2.5	8.7	0.099	Early carcinoma. Nephritis
47	63	31.5	18.2	3.7	9.4	0.327	Slow growing. Nephritis. Diabetic

TABLE I—*Continued*

NUMBER	AGE	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	AMINO ACID NITROGEN	SUGAR	REMARKS
Breast—Continued							
		mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent*	
48	36	28.0	11.2		7.3	0.104	Rapidly growing. Advanced
49	48	26.6	13.3		6.5	0.104	Recurrent. Metastases. Advanced
50	52	28.0	11.2		9.8	0.135	Advanced. Immunity
51	46	25.9	11.9		6.1	0.094	Short duration. Metastases. Rapid advances
52	43	22.4	9.1		7.4	0.135	Very malignant. Metastases in femur. Nephritis
53	45	29.4	20.3	2.9			Far advanced. Fair general condition
Mouth							
54	45	25.2	13.3			0.092	Superior maxilla. Long duration. Advanced
55	41	25.2				0.137	Inferior maxilla. Short duration. Malignant
56	40	33.6	15.4			0.091	Carcinoma antrum
57	60	28.0	12.6			0.115	Carcinoma inferior alveolar process. Advanced
58	56	30.8	18.9			0.113	Carcinoma superior alveolar process. Very malignant
59	42	23.8	13.3	3.2	5.3	0.115	Carcinoma inferior alveolar process. Advanced. Slough
60	60	34.6	15.4	4.2		0.099	Carcinoma superior alveolar process. Not very malignant
		34.3	16.4		6.0	0.110	Same case, 2 months later
61	56	30.8	15.4		6.3	0.145	Carcinoma superior alveolar process. Rapidly growing
62	63	40.7	19.6		8.2	0.109	Carcinoma floor mouth. Degenerative changes of age. Local, but not very malignant
63	35	30.8	15.0		6.0	0.084	Carcinoma inferior alveolar process. General condition fair. Advanced, but local
64	70	29.4	16.8		7.1	0.120	Floor mouth; local. Nephritis. Alcoholic

TABLE 1—*Continued*

NUMBER	AGE	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	AMINO ACID NITROGEN	SUGAR	REMARKS
				Mouth—Continued			
		mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent*	
65	58	25.9	13.6		7.9	0.102	Carcinoma inferior maxilla. Rapid and very malignant
66	63	27.3	17.5	<1		0.107	Carcinoma inferior alveolus. Rapid and very malignant
67	40	35.1	13.3		10.5	0.111	Carcinoma superior alveolus. Rapid and advanced
68	54	19.1	10.5		7.2	0.126	Floor mouth. Long duration. Far advanced. General condition bad
69	54	28.0	14.0				Early antrum. Rheumatism
70	75	37.9		3.0			Papillary carcinoma of buccal cavity. Senile changes
71	52	28.7	11.5	<1			Carcinoma superior maxilla. Local and slowly growing
72	53	36.5		2.3			Carcinoma floor mouth. Short and very malignant
				Prostate			
73	66	29.4	17.5			0.095	Long duration. Locally, far advanced. Alcoholic
74	66	32.2	19.6			0.100	Postoperative. Recurrence
75	69	30.8	14.0	3.1	5.9	0.137	Terminal stage. Chronic rheumatism
76	71	26.6	10.5	3.7		0.133	Rapid course
77	51	28.0	15.4		7.2	0.085	Rapid and very malignant
78	62	43.4	28.7	5.1	6.6	0.105	Short duration. Very malignant. Metastases. Kidneys
79	74	31.5	15.4		7.8	0.112	Long duration
80	78	28.0	14.3		5.5	0.125	Rapid and very malignant
81	72	44.2	21.0		8.4	0.105	Local and of long duration. Pyelitis
82	33	23.8	12.8			0.100	Extensive and very malignant. Sarcoma
				Rectum			
83	66	32.2	16.8	2.5	8.9	0.126	Rapid. Well advanced
84	46	22.4	9.8	1.5	7.6	0.084	Recurrent. Very malignant
85	39	28.7	12.3		8.7	0.105	Well localized. Tr. alb.

TABLE 1—*Continued*

NUMBER	AGE	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	AMINO ACID NITROGEN	SUGAR	REMARKS
				Rectum—Continued			
		mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent*	
86	37	30.8	15.4	2.4	9.0	0.090	Far advanced. Very malignant
87	40	30.8	14.0		7.8	0.149	Recurrent; slow growing; extensive
88	68	30.8	16.1		8.0	0.101	Local lesion. Well advanced
89	60	29.4	16.4		8.4	0.117	Recurrent. Long duration. Very malignant
90	54	18.2	9.7			0.084	Very malignant
91	62	29.4		<1			Postoperative. No visible carcinoma
92	52	31.5		1.8			Extensive. Malignant. Immunity
93	49	32.2		1.6	10.7		Short duration. Very malignant. Advanced
94	50	26.6	14.0	<1			Adenocarcinoma. Local lesion, not far advanced
95	50	35.1		2.6			Long duration. Slow growing
				Bladder			
96	62	30.8	17.9			0.100	Papillary. Short duration. Rapid growth
97	71	37.9	24.5	1.3	10.4	0.118	Advanced. Degenerative changes
98	71	30.8	16.8	1.7	5.6	0.072	Rapid course. Extensive lesion
99	31	23.1	9.8		6.2	0.118	Advanced. Very malignant
100	53	33.6	15.4	4.4	6.9	0.094	Well advanced
101	63	37.9	24.5	3.5	7.0	0.125	1 year duration. Kidneys
				Melanoma			
102	46	33.6		5.1		0.062	Terminal stage. Primary mole two years previous
103	35	29.4	14.0		8.6	0.111	Chest
104	58	30.1	17.5	4.5		0.112	Palate. Far advanced. Long duration
				Sarcoma, soft			
105	34	23.8	15.4			0.095	Pleura. Advanced. Immunity, Reacts to x-ray

TABLE I—*Continued*

NUMBER	AGE	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	AMINO ACID NITROGEN	SUGAR	REMARKS
Sarcoma, soft—Continued							
		mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent*	
106	61	30.1	12.9	1.8		0.091	Breast. Metastases to eye after 6 years
107	55	30.8	15.4		7.6	0.084	Testis. Advanced. Short duration
108	33	32.2	19.3	3.3	5.4	0.120	Abdominal wall. Albumen
109	47	26.6	13.3	3.2		0.110	Nasopharynx
110	30	35.1		1.8			Teratoma testis
111	56	25.2	10.5	1.8		0.112	Advanced
112	48	32.2	17.5			0.118	Choroid, locally advanced
113	67	30.1	16.8				Back. Local. Operable
114	52	30.1	15.0	<1			Testis. Anaplastic
115	47	24.5	11.9			0.115	Rapid and malignant
Lymphosarcoma							
116	47	30.1	13.3		4.7	0.120	Responded to treatment and then rapidly progressed
117	50	27.2	15.7		6.2	0.110	Rapid; died in 2 months
118	35	30.8	18.2	5.0			Bulky; not far advanced. Kidneys
119	52	35.1	15.4			0.070	Recurrence
Sarcoma, hard							
120	21	39.3				0.091	Femur. Advanced. Metastases in chest
121	44	37.9	23.8			0.104	Humerus. Terminal. Metastases in lung
122	39	21.0	12.9			0.107	Humerus. Myxoma. Very rapid
123	55	28.7	12.6	1.7		0.103	Fascial thigh. Very malignant. Sepsis
124	24	35.1	22.9		7.7	0.096	Occipital. Terminal stage
125	44	21.0	10.1	1.9	5.2	0.102	Atypical. Arm. Metastases in lung. Advanced
126	18	28.0	13.3		8.3	0.096	Recurrent. Toxins. Marked immunity

TABLE 1—*Continued.*

NUMBER	AGE	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	AMINO ACID NITROGEN	SUGAR	REMARKS
				Sarcoma, hard—Continued			
		mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent*	
127	34	31.5	14.7			0.144	Tibia. Long duration. Far advanced. Toxins
128	33	27.3	13.6		10.4	0.117	Myeloma humerus
129	20	26.6	12.6		6.5	0.117	Femur. Extensive. Long duration
130	18	24.5		<1	9.6		Superior maxilla. Recurrent. Toxins
131	18	22.4	11.2	1.8			Rib. Metastases
132	38	25.9		1.4			Tibia. Metastases to chest
133		23.8	12.2			0.112	Pelvis
				Neck			
134	65	30.6	12.3			0.146	Far advanced. Degenerative changes
135	22	28.0	13.3			0.099	Rapid. Very malignant sarcoma
136	33	26.6	12.1		8.4	0.117	Slow; extensive locally
				Tongue			
137	42	28.0				0.087	Very malignant. Syphilis
138	45	35.1	16.8			0.142	Very malignant. Kidneys
139	57	22.4	9.8		5.8	0.144	Very malignant. Extensive recurrence
140	63	30.1	14.0	4.2	7.7	0.090	Extensive lesion. Kidneys
141	58	24.5	12.2		3.2	0.116	Early lesion. Syphilis
142	48	30.1		2.2			Early lesion. Good immunity
143	48	23.1	12.6		7.6	0.123	Late and advanced. Very malignant
				Larynx			
144	50	33.6				0.130	Advanced lesion. Diabetes
145	38	28.7	19.6	2.1		0.084	Long duration; no generalization
146	25	25.6	10.5		7.6	0.093	Long duration; no generalization
147	40	35.1	22.4	2.5		0.090	Long duration; far advanced; starvation
148	50	29.4		<1	10.3		Short duration. Very malignant

TABLE I—*Continued*

NUMBER	AGE	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	AMINO ACID NITROGEN	SUGAR	REMARKS
Lip							
		mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent*	
149	70	33.6	17.6			0.097	Slow growing. Recurrent. Arteriosclerosis
150	65	30.8	14.7	3.0	7.4	0.086	Terminal stage
151	44	30.8	17.1		6.0	0.120	Localized. Early growth
152	65	35.8		1.9			Recurrent. Arterial changes
153	61	37.2		2.7			Local. Not advanced
154	56	28.7			12.2		Local. Not much infiltration
Stomach							
155	59	30.8	11.2			0.110	Far advanced
156	46	21.0	10.5	2.4	10.0	0.090	Advanced; rapid. No starvation
157	46	35.1	11.5		6.9	0.100	Advanced; rapid
158	48	40.4	22.1			0.103	Advanced; long duration. Negative urine
159	66	28.0	19.6	3.3	8.0	0.089	Advanced. Starvation
160	72	39.3		4.6			Moderately advanced
161	60	24.5		1.0			Long duration. Starvation
162	48	28.0	18.2				Short duration. Starvation
163	54	42.1	18.6	3.6			Well nourished. Duration 2 years
164	55	35.8	21.0	2.9	5.3	0.367	Long duration. Diabetes
165	50	148.8	119.3	6.0		0.153	Kidneys. Carcinoma stomach?
166	50	171.2		5.9			Kidneys. Carcinoma stomach?
Esophagus							
167	60	35.1	19.6	1.7		0.116	Long duration; localized
168	45	32.2	19.3		8.4	0.113	Long duration. Slow growing. Kidneys
169	53	30.1	16.4		6.7	0.120	Local. Well advanced
170	72				7.2	0.090	Starvation
171	52	30.8		1.7			Early lesion
Hodgkins							
172	50	39.3	26.6	5.7		0.101	Advanced nephritis
173	24	26.6	12.6		6.9	0.103	Axillary. Bulky lesion
174	36	25.2	12.2		8.4	0.095	Extensive. Duration 1 year
175	22	28.0	18.2		7.4	0.101	Advanced. Good general condition

TABLE I—*Concluded*

NUMBER	AGE	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	AMINO ACID NITROGEN	SUGAR	REMARKS
Tonsil							
		mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent*	
176	50	29.4	11.5			0.090	Terminal stage
177	50	30.1	15.8		8.2	0.099	Long duration. Good general condition. Immunity. Nephritis
178	51	28.7			8.8		Moderately extensive. Alcoholic
Miscellaneous							
179	46	37.9	15.5	4.2	10.3	0.070	Chronic lymphatic leukemia. Negative urine. White count 308,000
	46	37.9	23.1	2.5	9.2	0.100	Same case in 2 weeks. *x*-ray. White count, 136,000
180	37	50.5	23.1	5.8	18.4	0.105	Myelogenous leukemia. Negative urine. White count, 245,000
	37	50.5	31.5		9.9	0.125	Same case in 2 weeks. Radium. White count, 89,000
181	47	33.6	13.3		6.9	0.121	Lymphatic leukemia. White count, 245,000
182	59	47.7	21.0	3.2	14.9		Polycythemia; red cells, 7,872,000; hemoglobin, 105; white cells, 57,950
183	68	29.4	19.6			0.099	Carcinoma thyroid. Recurrent. Long duration
184	57	29.4	15.4	2.8	5.6	0.111	Epithelioma buttocks. Advanced. Very malignant
185	45	30.8	21.7	1.9		0.082	Mixed tumor of parotid. Not very malignant
186	59	28.0	13.6			0.090	Carcinoma thyroid. Metastases to stomach
187	27	33.6	17.5		7.8	0.135	Abscess of brain
188	50	29.4		<1	11.6		Carcinoma of pancreas. Good resistance
189	39	36.5		1.1			Brain tumor

and it is quite to be expected that these cases should show the most marked chemical variation from the normal. It is of interest in this connection to note that Folin (17) has reported abnormally low nitrogen figures for the blood of normal pregnant women. Dr. Wm. G. Lyle (private communication) has also noted very low nitrogen figures in the blood of pregnant women in investigations carried out at the Roosevelt Hospital. Cancer and pregnancy are related in that both involve rapid cell proliferation within the body, and it is of much interest to note the chemical similarity of the blood in the two conditions.[1] Mosenthal and Lewis (18) report a low urea value for 5 cases of anemia.

Looking at the 189 cases as a whole, we find that 23 per cent have a non-protein nitrogen content of 25 mgm. per 100 cc. or below, and only 31 per cent are above 30 mgm. Urea nitrogen is also low, 31 per cent of the cases having 12 mgm. or less per 100 cc. of blood. Fifty-five per cent of the cases have a urea nitrogen representing less than 50 per cent of the total non-protein nitrogen. Uric acid values appear to be normal in our cases, except when kidney complications are present. Exceptions to this statement are to be noted in two cases of melanoma in which the uric acid figures are high, although there were no kidney complications detectable. Amino acids are present in slightly increased quantity, as compared with the normal figures reported by Bock. The sugar values are practically normal, or at least our cases as a whole would average within normal limits. This conclusion is contrary to the findings of several previous investigators, but we believe that our longer series of cases demonstrates that where there is no kidney involvement or diabetes present the blood sugar figures in cancer are not abnormally high. Certainly there is no relationship between the severity of the disease and an increased blood sugar.

[1] Dr. A. F. Coca has found almost complete absence of urea from the tissue of some breast tumors which he examined. (Private communication.)

PATHOLOGICAL AND CLINICAL FACTORS

In the earlier portion of this paper we have called attention to the fact that interpretations of the chemical data obtained from the blood of cancer cases should not be made without reference to the age and general condition of the patient. It is also necessary to consider the location of the tumor or tumors, as well as special involvement of any organ due to other disease or to the degenerative changes of advancing age. We believe that these factors have been largely neglected in such studies as have hitherto been reported. In tables 2, 3, and 4 we have presented our findings in such a way as to try to bring out any relationship which might exist between the results of the chemical analyses and the various factors above enumerated. A brief discussion of the results of such classification follows.

TABLE 2

Average results according to age

NUMBER OF CASES	DECADE	NON-PROTEIN NITROGEN	NUMBER OF CASES	DECADE	NON-PROTEIN NITROGEN
11	20–30	27	46	50–60	30
26	30–40	26	30	60–70	33
45	40–50	29	10	70–80	33

Age. In table 2, 168 patients are classified with age as the sole basis of the classification, and the average figures obtained for the non-protein nitrogen are recorded for each decade. From this table it appears that in the period from twenty to forty years, age has no influence upon the non-protein nitrogen content of the blood. For the following decades up to seventy years there is a slight but steady increase in this nitrogen value. It is to be expected that the degenerative processes of age, which are so apt to involve the kidneys, even where such changes may not be detectable clinically, would influence the non-protein nitrogen of the blood so that this figure would be higher than for younger individuals. Whether figures similar to ours would be found in supposedly strictly normal individuals of like ages cannot be

TABLE 3

Averages according to age and location of tumor

NUMBER	AGE	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	AMINO ACID NITROGEN	SUGAR	UREA NITROGEN	AMINO ACID NITROGEN	LOCATION OF DISEASE
		mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent*	*per cent*	*per cent*	
1– 40	45	26.6	13.6	2.6	7.8	0.105	51.0	29.6	Uterus*
41– 53	45	27.4	13.1	2.9	7.7	0.105	47.6	26.8	Breast
54– 72	58	33.6	15.5	3.2	6.1	0.111	46.0	18.0	Mouth
73– 82	67	31.8	16.9	4.6	6.9	0.109	53.2	21.8	Prostate
83– 95	51	29.1	13.8	2.1	8.6	0.107	47.4	29.6	Rectum
96–101	58	32.4	18.6	2.7	7.8	0.104	57.5	24.0	Bladder
102–104	31	31.1	15.7	4.8	8.6	0.095	50.5	27.6	Melanoma
105–115	48	29.9	15.3	2.4	6.5	0.093	51.0	21.8	Sarcoma, soft
116–119	46	30.8	15.7	5 (1)	5.5	0.100	51.0	18.6	Lymphosarcoma
120–133	30	28.1	14.5	2.3	8.1	0.108	51.6	28.9	Sarcoma, hard
134–136	40	28.4	12.6		8.4	0.120	44.5	29.6	Neck
137–143	51	27.6	13.1	3.2	7.0	0.117	46.5	28.8	Tongue
144–148	40	30.5	17.5	2.3	8.9	0.099	57.3	29.4	Larynx
149–154	60	32.8	16.5	2.5	8.5	0.098	50.4	25.9	Lip
155–166	56	32.5	16.6	3.0	7.8	0.098	51.0	24.0	Stomach*
167–171	56	32.1	18.4	1.7	7.4	0.109	57.4	23.3	Esophagus
172–175	33	26.6	14.3		7.6	0.094	47.6	26.8	Hodgkin's†
176–178	50	29.5	13.7		8.5	0.094	46.5	28.8	Tonsil

* Excluding 2 cases.

† Excluding one case.

TABLE 4

NUMBER	AGE	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	AMINO ACID NITROGEN	SUGAR	UREA NITROGEN	AMINO ACID NITROGEN	LOCATION OF DISEASE
		mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent*	*per cent*	*per cent*	
8	40	24.6	11.7	2.4	7.6	0.095	47.5	31.0	Uterus
6	39	25.2	11.5	2.9	7.1	0.107	46.0	27.8	Breast
7	56	30.8	16.1		8.2	0.117	52.1	26.8	Mouth
4	58	26.6	13.1	3.7	6.3	0.100	49.3	23.9	Prostate
5	52	26.6	12.7	1.8	8.3	0.092	47.6	31.4	Rectum
3	54	28.3	14.8	1.5	5.9	0.096	52.4	21.0	Bladder
1	47	24.5	11.9			0.115	48.0		Sarcoma, soft
2	47	24.4	12.8	1.7		0.106	52.0		Sarcoma, hard

answered without far more extensive studies on such persons than are so far available.

In table 3, the average results of the chemical analyses are given in connection with the average age of the patient and the location of the tumors. Table 4 represents the average results in the particularly rapidly growing tumors according to the different organs affected. From these tables we find that the non-protein nitrogen values in uterine, breast, and tongue cancers are lowest, while mouth, stomach, esophagus, and bladder tumors show the highest values. In the case of each organ, numerous factors of importance in the interpretation of the results should be mentioned.

Uterus. In 40 cases of uterine cancers the average age is forty-five years. This is within a decade in which average non-protein nitrogen value of cancer cases as shown in table 2 is 29 mgm. per 100 cc. The average non-protein nitrogen value for the uterine cases is 26.6 mgm. per 100 cc. In this decade degenerative changes are in general a negligible factor, although pyelitis and kidney changes may often be found in the terminal period of the disease from bladder and ureteral involvement. Uterine cancer is a very malignant growth and often rapid in its progress. Distant metastases, however, are comparatively infrequent. The majority of the patients were in an advanced stage of the disease when the analyses were made. Case 36 (table 1) is of interest in this connection. The growth in this case was apparently limited to the cervix in a patient thirty-eight years of age and the non-protein nitrogen was only 16.8 mgm. per 100 cc. Complete retrogression and apparent cure of the lesion resulted from the application of radium. No other pathological conditions were found, except a moderate anemia. Blood analysis recently made in this case gives the high non-protein nitrogen values of 34.5 mgm.

Breast. In 13 patients with mammary cancer the average age is the same as in the uterine cases. The non-protein nitrogen value for this series is 27.4 mgm. per 100 cc. Most of these lesions were advanced, distant metastases were common, and in many the progress was very rapid.

Tongue. The average age in six cases of carcinoma of the tongue is fifty-one years. The decade non-protein nitrogen value is 30 mgm. per 100 cc. Syphilis and degenerative changes are common. These lesions of the tongue were extensive and perhaps more malignant than cancer in any other organ.

Mouth. (Exclusive of lip and tongue tumors.) Eighteen mouth cases have an average age of fifty-eight years with a decade non-protein nitrogen of 30 mgm. per 100 cc. The average non-protein nitrogen content is 33.6 mgm. per 100 cc. Alcohol, syphilis, and teeth infections made nephritis and diverse degenerative changes sufficiently frequent to explain the high non-protein nitrogen value.

In cancer of the *prostate* and *bladder* the average age is sixty-seven and fifty-eight years respectively and the average non-protein nitrogen 31.8 mgm. and 32.6 mgm. per 100 cc. The location of the disease, as well as the comparatively advanced age of these cases, makes pyelitis and kidney changes a frequent factor in the results of the blood analyses.

Stomach. In 11 cases of carcinoma of the stomach the average age is fifty-eight years. The decade non-protein nitrogen value for this age is 30 mgm. and the average non-protein nitrogen is 32.5 mgm. per 100 cc. All of the cases except two were far advanced. In two cases there is a very high non-protein nitrogen.

Rectum. The average non-protein nitrogen value in seven rectal cases is only a trifle less than the decade value. In all of the cases the progress of the disease was slow.

Sarcoma. Twenty-three cases of sarcoma of hard and soft tissues are all very malignant and rapidly-growing. The non-protein nitrogen value is 28.1 mgm. and 29.9 mgm. per 100 cc. respectively.

Lymphosarcoma. In three cases of lymphosarcoma, with an average age of forty-six years, the course of the disease was rapid. The non-protein nitrogen value is slightly higher than the decade value.

CONCLUSIONS

1. Non-protein nitrogen and urea nitrogen are in general low in the blood of cancer patients. Amino acid nitrogen is slightly above normal. Low results are not so obvious when other pathological conditions coexist.

2. Non-protein nitrogen and urea nitrogen of blood are consistently low in clinically malignant cases.

3. Uric acid, except in cases with kidney complications and in two cases of melanoma, is not abnormal in cancer blood.

4. Blood sugar is not generally increased in cancer. Twenty-six per cent (diabetics and nephritics excluded) show a figure somewhat above the normal and 13 per cent show a figure below normal.

REFERENCES

(1) Freund: Wien. med. Bl., 1885, viii, 268.
(2) Trinkler: Centralbl. f. d. Wissensch., 1890, xxviii, 498.
(3) Matrai: Pest. med.-chir. Presse, 1885, xxi, 629.
(4) Lewis and Benedict: Med. Rec., New York, 1914, lxxxvi, 650.
(5) Menten: Jour. Cancer Research, 1917, ii, 179.
(6) Luden: Jour. Lab. and Clin. Med., 1916, i, 662.
(7) Autenrieth and Funk: München. med. Wchnschr., 1913, lx, 1243.
(8) Bloor: Jour. Biol. Chem., 1915, xxiii, 317.
(9) Denis: Jour. Biol. Chem., 1917, xxix, 93.
(10) Greenwald: Jour. Biol. Chem., 1915, xxi, 61.
(11) Bock and Benedict: Jour. Biol. Chem., 1915, xx, 47.
(12) Marshall: Jour. Biol. Chem., 1913, xv, 487.
(13) Benedict: Jour. Biol. Chem., 1915, xx, 629.
(14) Neuwirth: Jour. Biol. Chem., 1917, xxix, 477.
(15) Bock: Jour. Biol. Chem., 1917, xxviii, 357.
(16) Lewis and Benedict: Jour. Biol. Chem., 1915, xx, 61.
(17) Folin: Jour. Am. Med. Assn., 1917, lxix, 1209.
(18) Mosenthal and Lewis: Jour. Am. Med. Assn., 1916, lxvii, 933.

ON SPIROPTERA CARCINOMATA AND THEIR RELATION TO TRUE MALIGNANT TUMORS; WITH SOME REMARKS ON CANCER AGE

JOHANNES FIBIGER

From the Anatomo-Pathological Institute of the University of Copenhagen

Received for publication, July 25, 1919

In this Journal (1918, iii, 227) Bullock and Rohdenburg have criticized some reports published during the last years, concerning the experimental production of malignant tumors (Fibiger, Yamagiwa and Ichikawa) and tumor-like formations (Stahr). Bullock and Rohdenburg emphasize that

> An analysis of the several reports mentioned reveals a number of assertions common to each, which are at variance with the recognized laws of malignant tumor growth, while, at the same time, it brings out points which cast a certain amount of doubt upon the authors' interpretation of their results.

In the following I shall reply to Bullock and Rohdenburg's criticism, though only in so far as it concerns the investigations on Spiroptera carcinoma communicated by me. I can so much the less avoid occupying myself with the assertions of these authors as their untenability seems to some extent to be due to an insufficient acquaintance with or a misapprehension of the details of my investigations of 1913 and 1914 (1–4). My most recently concluded investigations (8–11), which corroborate the results of the former, were not published until after the appearance of the authors' paper and so could not be known to them.

Bullock and Rohdenburg base part of their criticism on a comparison between the result of my investigations on Spiroptera carcinoma and the result of investigations carried out by them-

selves on the effect of mechanical, chemical, or mechanico-chemical irritants[1] on the stomach of the rat.

As a result of the investigations Bullock and Rohdenburg, in several passages as also in the summary of their paper emphasize:

1. That "Chemical or mechanico-chemical irritation of the squamous epithelium of the rat's stomach results in localized papillary tumors suggesting the cancroid growths produced by nematode worms."

Against this it must be pointed out that none of the figures accompanying the authors' paper has any real likeness either to epitheliomata (cancroids, carcinomata) in general or to the Spiroptera carcinomata produced by me and given in my plates, but only shows changes that cannot be designated as anything but simple hyperplasia and hyperkeratosis of the squamous epithelium of the fundus of the stomach of the rat with down-growth of the epithelium, combined with slight papillary transformations of the mucosa. Nor is it evidenced by the text that the authors have observed changes of genuine carcinomatous type.

On the other hand I will not deny that the changes produced by the authors accord with non-carcinomatous changes of similar kind produced almost constantly by the invasion of Spiroptera neoplastica (*Gongylonema neoplasticum*) (5) in the fundus of the stomach of the rat, disregarding the fact that these latter changes are often more pronounced. That changes of this kind are the usual effect of Spiroptera infection on the fundus of the stomach in rats and mice has been described in detail in my papers of 1913 and 1914 (1–4) the first of which contained numerous photographs showing what these changes were like. In all the 111 rats dealt with in these papers, in which the fundus of the stomach was at all pathologically transformed, such changes were demonstrated, and in 92 out of the 111 rats the changes were only of this type.

But it must be sharply emphasized that changes of the character here mentioned (which I have also had occasion to observe

[1] The irritants employed were spines of pig bristles, pin points, Scharlach R-oil, pine tar, pine tar oil (see the original paper).

may be produced from other causes than Spiropteræ) have not in a single case been looked upon by me as carcinoma-like or as true carcinomata, but have been regarded exclusively as mentioned above, i.e., only as hyperplasia, hyperkeratosis, benignant down-growth and heterotopy of the epithelium, combined with inflammation and frequently also with papillary transformation of the mucous membrane.

If in my investigations I had only been able to produce changes of this category any talk of a real Spiroptera carcinoma would have been entirely excluded.

When, however, it can now as little as hitherto be doubted that real carcinomata, morphologically possessing the specific characters of malignant epitheliomata, can be produced by Spiroptera infection, this is due to the observation of phenomena of an entirely different kind.

As will be seen inter alia from detailed descriptions in my previous papers from 1913 and 1914 (1–4) in a considerable number of Spiroptera infected rats, together with the above mentioned simple epithelial hyperplasia, more or less pronounced epithelial changes of quite another type occur, and this type has the specific character of the malignant squamous-celled carcinoma in pronounced shape. Changes of this kind were found in 19 out of the above mentioned 111 rats, combined with simple hyperplasia, and in later investigations (8) concluded in 1918, I have further demonstrated such real carcinomatous changes in 65 rats.

In order to throw light upon the peculiarities which characterize these changes and which entitle us to regard them as true carcinomata I shall quote as follows from one of my latest papers (1918) (8):

I have only felt entitled to fix the diagnosis of carcinoma in such cases as exhibited—through a series of sections—the following changes:

1. Heterotopical downgrowth of epithelial cells belonging not only to the normal type of the basal epithelial layers, but mixed with atypical and keratinized cells in abundance partly arranged as spherical masses and horny globes.

2. Infiltrative growth of these heterotopical and atypical epithelial cells into deeper layers, splitting up invasively the elements of the connective tissue of mucosa and the muscle cells of the muscularis mucosæ, forming isles and spurs in the latter or—as most frequently seen—also penetrating through this membrane into the superficial or deeper layers of submucosa.

And that these changes described by me as genuine carcinoma show the very closest agreement with the well-known changes in fully pronounced and typical squamous-celled carcinomata (malignant epitheliomata) in man and animals will be further evidenced by the plates accompanying my papers (see e.g., in my paper of 1913 (2), plates XI–XV, figs. 73, 78, 79, 83–85; in my paper of 1914 (4), figs. 15, 16, and others; also photographs in my papers of 1918 (8)).

What has been here adduced will sufficiently demonstrate that in reality—in opposition to the opinion of Bullock and Rohdenburg—there exists no similarity, but on the contrary, considerable divergence, between the hyperplasia produced by these experimenters and the changes characterized by me as real carcinoma, which in Spiroptera infected rats (frequently in black and white rats) occur together with epithelial hyperplasia, hyperkeratosis, downgrowth of the epithelium, and papillary transformation. These latter changes, which show considerable likeness to those produced by Bullock and Rohdenburg, must be sharply distinguished from the really carcinomatous changes, and, as already mentioned, have never been regarded by me as carcinoma-like or really carcinomatous.

To what degree I look upon the simple benignant hyperplasia as different from the real carcinoma is evidenced by my latest papers (of 1918) (10). Referring to the arguments there adduced, I shall here restrict myself to emphasizing that I regard the two forms of change, the simple epithelial hyperplasia and the real carcinoma occurring side by side in the fundus of the stomach in Spiroptera infected rats, not only as being morphologically but also as fundamentally different, mutually independent, simultaneously occurring effects of the same etiological factor, the Spiroptera infection.

The Spiroptera infection in all kinds of rats and mice hitherto examined by me produces inflammation, epithelial hyperplasia, hyperkeratosis, downgrowth of the epithelium, and papillary changes in the squamous-cell part of the stomach. Together with these more or less extensive changes, pronounced carcinomatous processes were observed in black and white rats in more than 50 per cent of the experimental animals. In wild house rats I have hitherto observed carcinomatous changes in only a single case, in white mice only in 3 cases. In wild Norway rats, house mice, and forest mice, I have not as yet observed carcinoma, though epithelial hyperplasia and downgrowth in these animals often reaches an enormous development, exceeding what may as a rule be observed in black and white rats. The investigations on the effect of the Spiropteræ on the various rodents have, however, not yet been concluded, and only as regards the black and white rats and the white mice comprise a considerable number of animals.

2. Bullock and Rohdenburg further point out that the Spiroptera carcinoma in the fundus of the stomach of the rat does not invade the muscularis or the glandular portion of the organ.

As a matter of course this circumstance does not really involve any objection whatever to the diagnosis of carcinoma, granting that the observed changes otherwise possess the fully typical properties of the carcinoma. Like all other carcinomata, the Spiroptera carcinoma requires a certain time to spread ad maximum, and in a greater number of the cases observed by me the explanation why the muscularis had not been invaded and the process had not penetrated into the glandular portion must simply be sought in the fact that the animals died so early that the process had not yet reached its greatest extension.

This explanation is quite satisfactory in all the cases where the Spiroptera infected rats died one and one-half to three months or less after transmission of the Spiropteræ, and the Spiroptera carcinoma had not extended to the deep layers of submucosa. But not even in other cases, where the rats had survived the transmission of the Spiropteræ for a longer time (maximum eight to nine months), and the carcinoma had invaded large areas of

the deepest layers of submucosa, did I ever find the muscularis carcinomatous, not even in cases where the carcinoma had produced metastases.

The reason for this might, of course, be found in these cases also in the fact that the rats concerned had succumbed so soon to the Spiroptera affection or complicating diseases that the carcinoma in the fundus of the stomach had not yet reached its greatest development. For it must be recollected that the Spiroptera affection, whether it consists only of papillary transformation of the fundus, or whether this change is further complicated by carcinoma, is in any case an exceedingly serious disease, which is bound to weaken the nutrition and digestion of the rats and to a great extent shorten their span of life.

Now it cannot be doubted that black and white rats, which have chiefly been employed in the investigations, are but little resistant, whether the Spiroptera affection of the fundus of the stomach is carcinomatous or not, since they are not able to survive the infection for such long periods as other rats or mice, but succumb comparatively early; sometimes, it would seem, from the Spiroptera disease alone, but frequently also from infectious complications (especially pneumonias). But of Spiroptera infected wild Norway rats, house rats, house mice, and white mice, not only a large number have proved able to survive the infection for a longer time, but many Spiroptera infected animals of these species have been able to live much longer than the average period for black and white rats.

For the elucidation of the question, whether the escape of the muscularis in carcinoma of the stomach in Spiroptera infected black and white rats should be sought in their shorter term of life, it will therefore be of interest to inquire into the behaviour of the carcinoma in the more resistant species. As mentioned above I have hitherto only found Spiroptera carcinoma in the fundus of the stomach in one house rat and 3 white mice, and in 2 of these cases (in the house rat and in a white mouse) the downgrowth of the carcinoma was of comparatively slight extent. But the two other cases observed in mice offer greater interest; one of these will here be reported, whereas the other has been previously (11) communicated in detail.

Mouse I. Six weeks old albino male mouse, fed January 12, 1917, on muscles from the femora and prothorax of 3 cockroaches (*Periplaneta orientaiis*) infected with Spiroptera. Upon examination of the excrements of the mouse, typical Spiroptera eggs were demonstrated on July 30, 1917. The mouse died February 26, 1918, about fifteen months old, 410 days after the transmission of the Spiroptera. Its weight at death was 15 grams.

Post mortem examination shows diffuse purulent peritonitis. The stomach measures rather more than 1 cm. from side to side and about 7 mm. from top to bottom. The fundus is greatly thickened and irregularly knotted, especially at the greater curvature. At the fore and top part of the latter, where the thickening is most pronounced, the cause of the peritonitis is found in a complete perforation of the wall at a projecting portion, near which the stomach adheres to the posterior surface of the liver.

The tongue and esophagus exhibit no special changes. The right lung contains a sharply marked-off white nodule, rather larger than a hemp seed, part of which is at once taken out, cut to pieces, and transplanted intraperitoneally in 10 young white mice weighing 8 to 10 grams. The organs otherwise normal and without metastases. The wall of the fundus shows papillary transformation in spots, especially in the perforated portion, the thickness of which is 4 to 5 mm. In the fundus, the epithelium is everywhere strongly desquamating and hyperplastic. Compact epithelial columns penetrate downwards here and there, piercing the submucosa which, like the connective tissue of the mucous membrane, shows more or less proliferation and infiltration with lymphocytes and leucocytes.

Over an area of about 4 mm., answering to the macroscopically demonstrated perforation, pronounced carcinomatous formation is found, atypical epithelial projections mixed with numerous epithelial pearls and horny globes penetrating heterotopically downwards and infiltratively invading the mucous membrane, submucosa, and muscularis. Both the inner and outer layers of this membrane are invaded by the carcinoma, which penetrates into the bundles of muscular fibrillæ, splitting these and extending further into the serosa, which shows advanced inflammation and adheres to the liver in a considerable area. The perforation has taken place through the central part of the carcinomatous portion, which shows strong necrosis of the carcinomatous masses. The surface epithelium everywhere, both in the carcinomatous part of the fundus and in the other portions, contains

numerous sections of Spiropteræ as well as ripe eggs. The pyloric portion is normal. The adjacent lymph-nodes do not contain metastases. On the other hand, the nodule demonstrated in the right lung, as beforehand assumed, proves to be a metastasis built up of typical squamous-cell epithelium, keratinizing cells, and horny globes that fill out numerous alveoli. The bronchial epithelium is normal, nowhere metaplastically transformed, and has no connection with the metastasis. The metastasis is, however, over no small area in slight or pronounced necrosis, which may perhaps be the cause of the negative result of the transplantation experiments.

The case here communicated proves that the Spiroptera carcinoma in the fundus may very well invade not only the submucosa but also the muscularis and the other part of the stomach wall, and the other case previously communicated in detail, which I shall return to in the following, shows the same. In these two cases the experimental animals died respectively 410 and 482 days after the transmission of the Spiropteræ. For comparison, it may be stated that the period of survival after transmission of the Spiroptera for carcinomatous black and white rats, at the utmost, and only for a smaller number of rats, has been 250 to 298 days. Hence the possibility cannot be ignored that the less deep penetration of the Spiroptera carcinoma into the stomach wall in black and white rats has—as mentioned above—only been a consequence of the fact that these rats have, on the whole, survived only for a short period the Spiroptera infection, and have therefore succumbed at a time when the carcinoma had not yet had sufficient time to penetrate into the muscularis.

But other factors also may possibly have played a part. The difference of the frequency of Spiroptera carcinoma in rats and mice pointed out in a preceding paper (10), indicates that differences not only according to individuals, but also according to species, and probably according to race, too, exist in the susceptibility to the cancer-producing power of the Spiroptera. Whether or not similar differences also exist in the power of resistance to the invasive growth of the carcinoma into the deepest layers of the wall of the fundus of the stomach, is a problem which cannot as yet be definitely solved.

That Spiroptera carcinoma possesses the power of invasive growth in muscular tissue will again appear from observations other than those mentioned above. As I have communicated in detail in a paper of 1918 (9), the Spiroptera invasion may also cause a development of carcinoma in the tongue of rats. The photographs of the 5 cases which accompany my paper, will show the occurrence of a very considerable, heterotopical, intermuscular, carcinomatous downgrowth, which in two cases reached such a degree that the muscles of the tongue were invaded for one third or one half of their entire thickness. Metastases to lymph-nodes or to other organs could not be demonstrated.

But in two cases the carcinoma was found to invade the perineural lymphatic spaces, so that these latter were filled out to a considerable extent with typical carcinomatous cells, and with epithelial pearls and horny globes enclosing the nerves like a sheath.

3. Bullock and Rohdenburg furthermore emphasize that "the presence of metastatic growths remains after all unproved," and add that "one of the appearances" given in my photographs might be "a heterotopic epithelial area like that sometimes seen in the peritoneal nodes of man." I presume that the authors are here referring to a metastasis in a lymph-node described and photographed by me in my first paper (2).

With regard to this observation I submit the following statement: In a black and white rat the fundus of whose stomach contained a strongly developed Spiroptera carcinoma, in a retroperitoneal lymph-node 1.5 cm. long, 8 mm. broad, and 0.5 cm. thick, situated close to the spleen, two almost spherical, sharply marked-off, white, firm nodules were demonstrated, the diameter of which was about 1.25–1.5 mm. Upon examination in serial sections these nodules proved to be built up partly of epithelium of exactly the same type as the deeper layers of the carcinomatous cells in the fundus of the stomach, and, especially in the central part of the nodules, partly of strongly keratinizing flat epithelial cells. It was beyond all doubt that this was a typical carcinomatous metastasis in a lymph-node (see figures) (2) and it is entirely precluded that a "heterotopic epithelial area" should here be present. I should be greatly interested if the authors would give further details of the occurrence of

such "heterotopic" keratinizing squamous-cell areas in the lymph-nodes of rats. Personally—in spite of the examination through years of a very large number of peritoneal lymph-nodes—I have never observed phenomena of a similar character to those here mentioned either in man, rats, or mice.

Bullock and Rohdenburg further emphasize that I have not differentiated a pulmonary metastasis from a form of bronchopneumonia which has been described in rats and is characterized by the presence of a squamous epithelium through metaplasia of the bronchus. The authors speak only of "his pulmonary metastasis" as if I had only once observed phenomena of this kind, though in my paper (of 1914) (4) quoted by them, I give an account of two corresponding observations and my first paper (from 1913) (2) contains one. I suppose the authors refer to the observation communicated in this first paper (1913), about which I shall further state that, as, indeed, it appears from the accompanying photograph, distinct spherical nodules were present (greatest diameter about 0.8 mm.), well marked off from the bronchial epithelium, composed partly of closely packed masses of epithelial cells showing the same structure as that of the deeper layers of the carcinomatous cells in the stomach wall, partly of epithelial cells in which very perfect keratinisation had taken place; horny globes also were found. In serial sections neither was epithelial metaplasia discoverable in the bronchus and the broncheoli, nor did the surrounding areas of the parenchyma contain any bronchopneumonia. If in my earlier description of this observation I did not mention that metaplasia of the bronchial epithelium (as described, e.g., in mice by Tyzzer in 1909) might be precluded, the reason was, of course, that in the present case there could be no question at all of changes of such a nature. The lesion was a typical intravascular metastasis. Besides, the metastatic nature of these changes, as well as of the changes of the lymph-node mentioned above, has been generally recognized.[2]

[2] Preparations of these metastases have been shown at lectures in Copenhagen (The Medical Society, 1913), in Bruxelles (La troisième Conférence internationale pour l'étude du Cancer, 1913), in Berlin (Berliner medizinische Gesellschaft, 1913), in Kristiania (Meeting of Scandinavian Naturalists, 1916), and elsewhere.

That Spiroptera carcinoma may produce metastases in the lungs will appear not only from the preceding observation of Spiroptera carcinoma in a mouse, but from later observations in rats. By an exhaustive examination of both lungs in toto in serial sections in 5 white and black rats with primary carcinoma in the fundus of the stomach, I have found small nodules of a structure corresponding to that mentioned above, without it being possible to establish any metaplasia whatever of the bronchial epithelium. Nor has it ever been possible, in spite of the examination of lungs from numerous other, non-carcinomatous rats, to demonstrate changes showing a structure similar to these metastases, though in some few animals metaplasia of the bronchial epithelium was demonstrated. In all, metastases in the lungs have thus been observed in 8 cases. Furthermore, peritoneal metastases of a Spiroptera carcinoma in a mouse (II) will be described below.

Metastatic growths in the perineural lymphatic spaces have already been discussed.

4. As an objection to the Spiroptera carcinoma, Bullock and Rohdenburg point out that it is not stated definitely, "whether continuous irritation is necessary for continuous growth and whether a type of proliferation can be inaugurated that no longer depends upon the presence of the irritant."

With regard to this objection, I shall call attention to the fact that I have previously shown that the metastases of the Spiroptera carcinoma do not contain Spiropteræ, and to the results of a series of investigations published in 1918 (10). In my first investigations (1913–14), I observed that as a rule several or numerous Spiropteræ were discernible in stomachs which exhibited pronounced changes, while stomachs with slight or doubtful changes would harbour only a single or a few parasites. At the same time, however, I laid stress upon an exception to this rule; thus in certain cases the stomach will contain but a few nematodes in spite of pronounced carcinomatous changes, a fact which I ascribed to the original considerable number of Spiropteræ having been diminished by a later emigration or dying off, analogous to the emigration of other species of nematodes, e.g., ascarides.

Renewed examinations, continued during the years that have passed since these original investigations, have shown that such an emigration plays a far more prominent part than I supposed at first. Referring the reader to one of my previous papers (1918) (10), in which all particulars are to be found, I shall only point out here that it is now beyond all doubt that in rats infected with the Spiroptera a considerable emigration of worms from the fundus of the stomach will take place in numerous cases when a period of three months or more has passed after the transmission of the worms. The number of Spiropteræ in the stomach, even as early as three months after the transmission of a great number of the parasites (several hundreds), may be so reduced that the total epithelial covering contains altogether but one or two or a few parasites. This observation now becomes worthy of special interest by the fact that the frequency of carcinoma of the stomach among the longest lived rats—as will be seen in my paper (10)—proved to be about the same, no matter whether the stomach contained a great or a small number, or only one or two or a few worms.

Entirely corresponding observations can be made in mice. In mice which had survived the transmission of a great number of Spiropteræ for a long time, I have often found the cardiac portion of the stomach to contain only a single or a very few Spiropteræ, and in the advanced carcinoma of the stomach of a mouse (II), mentioned below, which died 482 days after transmission of the Spiroptera, no nematodes were macroscopically traceable by post mortem examination, and only by microscopical examination did I succeed in finding a single specimen. But that the fundus of the stomach had actually harbored egg-producing parasites for a period of at least eleven months was evidenced by the fact that the excrements of the mouse during life had contained Spiroptera eggs 322 days after the transmission of the worms. In this case the carcinoma had continued its vigorous development and produced large peritoneal metastases, in spite of the nearly complete disappearance of the Spiropteræ.

Quite corresponding observations are also met with in Spiroptera infection of the tongue. Among the 5 rats, in the tongue of which Spiroptera carcinoma was produced, only 3 harbored the Spiroptera in the epithelium of their tongues, whereas the worms were entirely wanting in the tongues of the 2 longest lived of these rats, which had survived the transmission of the parasites from $5\frac{3}{4}$ to $6\frac{3}{4}$ months. And in the simultaneously developed carcinomata of the stomach of these 2 rats, only a very few specimens were to be found.

These observations show that the primary Spiroptera carcinoma in the fundus of the stomach of rats and mice and in the tongue of rats, as in the metastases, being once produced, is able to subsist and continue developing even if the irritant—viz., the Spiropteræ—which originally induced it partly or completely disappears. As to the conclusions which may be drawn from these and other analogous observations, I shall refer the reader to my detailed report, and merely point out that the observations here mentioned prove that no significance can be attached to the objection of Bullock and Rohdenburg quoted above.

5. On the other hand, these authors are perfectly right in emphasizing that transplantation of the Spiroptera carcinoma has been unsuccessful in earlier experiments. It cannot, however, surprise any one that the experiments have given negative results if we consider: (1) that, as is generally known, great difficulties often occur in the propagation of strongly keratinizing tumors; (2) that, owing to technical difficulties, I may not, in all experiments in which I have been unable to inoculate any greater number of young rats, have succeeded in getting a sterile material for inoculation from the fundus of the stomach (or from the tongue); (3) furthermore, transplantation of metastases has been precluded, these secondary deposits having in most cases been of such tiny dimensions that only microscopical examination could establish their existence.

From the two mice mentioned above a suitable amount of metastatic deposit could be obtained for the first time. However, transplantation of the metastasis in the lung of mouse I gave no positive results. More successful were the transplan-

tations from mouse II mentioned above, of which only a short account will be given here, a complete record of the case having appeared in my special paper (11) in which further particulars are to be found.

Mouse II. Albino male mouse six to eight months old, fed on February 16, 1916, on muscles of 5 cockroaches (*P. americana*), infected with the Spiroptera. On January 3, 1917, typical Spiroptera eggs found in the excrements of the mouse. The mouse died on June 12, 1917, 482 days after the transmission of the Spiropteræ. The weight at death was 24 grams.

The abdominal cavity is widely distended by an irregular tumor of the size of a large hazel nut, taking up the place of the stomach, and in the most lateral left portion adherent to the left lobe of the liver, to the left side of the diaphragm, and to the upper pole of the spleen. Tumor measures 2.5 x 2 x 1.5 cm. It seems due to an enormous new growth, partly in the stomach wall itself, partly perforating the latter, tumor masses being visible on the outer side of the stomach wall as well. The pyloric portion is apparently undamaged. The mouth cavity, tongue, and gullet are normal. In the mesentery, the omentum, and the peritoneal cavity, especially in the dorsal wall of the latter, numerous isolated, greyish-white, firm, roundish tumor nodules as large as the head of a pin up to the size of a pea are found. All the rest of the internal organs are normal; no metastases. No cutaneous or subcutaneous nodules. The wall of the fundus of the stomach proves to be about 0.5 to 1 cm. thick and consists of homogeneous greyish-white tumor tissue in which pronounced necrosis has taken place on the cavity side. The mucous membrane of the pyloric portion seems to exhibit no special changes; the line of demarcation towards the fundus is, however, hardly discernible. The stomach contains neither concrements nor hairballs. The intestines present no abnormalities.

On microscopical examination the greatly thickened part of the stomach wall is found to be the seat of a typical and extensively necrotic squamous-cell carcinoma with numerous areas of keratinisation. In most places the carcinoma extends to all the membranes of the wall, lining the cavity with a necrotic tissue and reaching not only into the serosa but penetrating this membrane and in great areas invading the adherent superficial parts of the liver, the spleen, and the diaphragm, the muscular bundles of which are split up by carcinomatous tissue.

In the wall of the fundus a single fully developed Spiroptera is found. The metastases all prove to be typical more or less keratinizing carcinoma, of the same structure as that of the cardiac portion.

Transplantation of the tumor was effected in immediate continuation of the post mortem examination. Fragments (weighing about 10 to 25 mgm.) of a peritoneal non-necrotic metastasis were used for the implantation. At the 1st transplantation, 12 white mice, weighing 10 to 15 grams, were inoculated, 7 subcutaneously and 5 intraperitoneally.

In 4 of the 7 mice inoculated subcutaneously, and in all mice inoculated intraperitoneally, tumors developed. The transplantations were continued, and in all the tumor proved transplantable during half a year for 4 generations. Taken together the transplantations gave positive results in 28 out of 55 inoculated mice which did not die immediately after the implantation. The tumor was preserved living by means of transplantation for altogether twelve months.

The inoculated particles very quickly reached a considerable size, weighing 9 to 11 grams, in several animals which died two to three months after the transplantation and being as large as a hazelnut, a walnut, or a plum.

Like the original tumor, the transplanted tumors exhibited the structure of a typical, keratinizing, squamous-cell carcinoma and kept their histological features almost unaltered through all 4 generations. They contained neither Spiropteræ, parts of Spiropteræ, nor eggs.

In several mice they grew invasively into the muscular tissue and into adjacent organs.

Again referring the reader to my recently published paper (11), in which the experiments here briefly summarized are communicated in extenso with all details and illustrations, I shall here restrict myself to emphasizing the main result of the experiments: the transplantability of the Spiroptera carcinoma.

6. Finally, Bullock and Rohdenburg allege as an essential argument against classing the Spiroptera carcinoma as a true malignant neoplasm, that the age of the animal is a negligible

factor in the incidence of these carcinomata, young animals being almost as susceptible as old ones.

In answer to this it must, however, be observed that no grounds for such an assertion are found in my paper of 1914 (4) quoted by the authors. In this it was merely pointed out that the Spiroptera carcinoma may also develop in quite young black and white rats, carcinoma in the fundus of the stomach having been demonstrated by me in a rat which, upon transmission of the Spiroptera, weighed only 30 grams, and at death (120 days later) 100 grams. The authors have, however, a greater justification for their conception of my opinion with regard to the occurrence of the Spiroptera carcinoma in young animals than can be gathered from my above mentioned paper, in so far as in later investigations (10) I have not been able to demonstrate with certainty a greater frequency of the carcinoma amongst unquestionably older than amongst unquestionably younger animals, and Spiroptera cancer occurred in rats which, both on the transmission of the Spiroptera and at death, were still young.

It has, however, proved impossible in these experiments to realize an exact estimation of the age of the rats and I have therefore felt bound to point out (10) only that the age of the animals is not likely to have been of any great importance.

But even if, through continued experiments, it should be established with certainty that Spiroptera carcinoma develops with the same frequency in young animals as in older ones, this cannot be advanced as an argument against the carcinomatous nature of this tumor. I do not agree with Bullock and Rohdenburg, either, when they declare "If we accept these tumors as malignant neoplasms, we must modify the current view concerning a cancer age, which has hitherto been held applicable to animals as well as to man;" or when they continue "We must conclude, in other words, that rats and rabbits differ in this respect from man and from the mouse, in which Murray, Slye, and others have shown that age is a very important factor for the origin of carcinoma."

The foundation for this generally accepted opinion here called attention to by the authors is, of course, the fact that carcino-

matous tumors in man and animals belonging to higher age periods are observed with a frequency which far exceeds the frequency of the relatively rare cases in which carcinoma can be demonstrated in young individuals.

It is, however, far too often forgotten that in determining the age at which man is attacked by carcinoma we can, as a rule, only take the date at which the carcinoma manifests itself as a demonstrable tumor; that this date is not tantamount to the real date of the first origin of the tumor is so obvious that any argument in support thereof is unnecessary.

This is of course also the case, and to a still greater degree with regard to the determination of the origin of tumors in animals. That tumors are almost exclusively found in older mice or rats really means only that tumors which have reached such a size that they are palpable or visible to the naked eye or on inspection with the lens are preeminently found in older animals; but it tells us nothing about the length of time in which these tumors have been present in non-demonstrable state, so that the real date of their earliest origin cannot be determined.

I must therefore dispute the correctness of the assertion advanced by Bullock and Rohdenburg, that investigations of tumors in mice and rats, however great the importance we must assign to them, have given more conclusive evidence of tumors preeminently occurring in older individuals than the evidence long since produced by clinical experience from man.

That the current opinion about the date of origin of the tumors is not often very far wrong must, however, be considered probable, as a considerable antedating of the origin of the tumors would only be justified on the improbable assumption that malignant tumors usually grow slowly.

But the rule that tumors occur preeminently in older individuals is not without exception, as is commonly known. Undoubtedly the number of true observations of malignant tumors in children has been greatly diminished through the critical examination of Philipp; still, a series of cases must be considered trustworthy. Certainly, incipient malignant tumors are sometimes found in young individuals dead from other diseases, but

it cannot be doubted that such tumors actually occur with greater frequency in the young than is generally assumed.

As it is well known, the war has caused the question of the frequency of carcinoma in soldiers (i.e., younger men) to be taken up again for discussion, and some investigators have observed carcinoma with astonishing frequency. As a control, Waetzold (12) has further summarized the number of carcinomatous individuals in the German army in ten years before the outbreak of the war, and in this period has found no less than 53 cases in men under thirty-five years (amongst which are 32 cases in soldiers of the age of nineteen to twenty-five years). Waetzold points out that the current opinion that carcinoma is a disease belonging to mature age (that is the years from forty to fifty) will no longer hold good.

Furthermore it must not be forgotten that some sorts of carcinoma develop with equal frequency in younger and in elder individuals, perhaps even with greater frequency in younger. Thus the common occurrence of Röntgen ray carcinoma in young men is a well established fact. Observations on the Bilharzia carcinoma, furthermore, indicate that this form of cancer also does not specially attack older individuals.

Hence, the power of the Spiroptera carcinoma to develop not only in old but also in young animals is a fact which is by no means without analogies among the tumors of man.

And for this reason Bullock and Rohdenburg's use of this fact as an argument against the classing of the Spiroptera carcinoma among the true neoplasms is quite unjustified.

But even if, in spite of all uncertainty, we might really venture to take it for granted that the majority of carcinomata in man and animals only begin their development in individuals that had reached an advanced age, we are not therefore justified in considering a more advanced age as, in the strict sense of the word, a truly predisposing factor in the development of such tumors. As is generally recognized, chronic irritation is a very important factor in the pathogenesis of numerous tumors. Their predominant occurrence in a developed state in older individuals need not, then, in itself mean anything but that most of the etiological

factors, in order to produce tumor at all, would have to act for such a long period that the tumors could not possibly appear except in individuals who for a great number of years had been exposed to the influence of the factor or factors in question and eo ipso must belong to older age-classes.

And the cause of the late appearance of the cancer might further, with regard to certain forms of cancer, be this, that only adult individuals were at all exposed to the etiological factors that produce these tumors, and that their occurrence would thus be postponed till a later age.

If, for example, we must assign a certain significance to the abuse of alcohol in the etiology of esophageal carcinoma, as generally assumed, the almost exclusive occurrence of this carcinoma in men past forty years of age could be accounted for simply by the fact that children are very rarely drunkards. And conversely, the frequent early occurrence of Bilharzial carcinoma might be a consequence of the fact that Bilharzial infection often takes place in childhood.

On the other hand, the frequent occurrence of Röntgen ray carcinoma in young men can hardly be explained analogically. Here the cause must rather be assigned to the fact that this irritant acts with greater intensity than the majority of irritants commonly in action, and is so vigorous in its effect that in a considerable number of individuals it is able to produce dermatitis and cancer of the skin even after a few years.

That Spiroptera carcinoma may occur in young animals might, perhaps, be accounted for in like manner.

From what has here been stated alone, it will appear with sufficient clearness that the generally accepted doctrine that "cancer age" must be taken as a proof that an advanced age in itself—as a special constitutional property of the organism—is a predisposing factor in the development of cancer, is in need of revision.

As a real proof of the existence of an age-predisposition in the strictest sense of the word, it would be required that older animals exposed for equally long periods to the same effective influence as young animals, more frequently become carcinomatous than the latter. But such a proof does not exist.

SUMMARY

By transmission of *Spiroptera neoplastica* (*Gongylonema neoplasticum*) to black and white rats and white mice, the development of neoplasms can be induced in the fundus of the stomach, and in rats in the tongue also. These neoplasms possess exactly the same histological structure as malignant epitheliomata (keratinizing squamous-cell carcinomata) in man and animals. They grow invasively into connective tissue and muscular tissue, and produce metastases in lymph-nodes, perineural lymphatic spaces, the lung, and the peritoneum. They continue their growth whether or not the Spiropteræ (as observed in the tongue) disappear entirely or only partly. They are transplantable, and when transplanted grow invasively into organs and tissues. Neither the metastases nor the transplanted tumors contain Spiropteræ, which have no share in their development and growth.

That these tumors are true carcinomata cannot, thus, be doubted, and the fact that they may occur in younger animals does not diminish our right to range them among the true malignant neoplasms.

REFERENCES.

(1) Fibiger, Johannes: Recherches sur un nématode et sur sa faculté de provoquer des néoformations papillomateuses et carcinomateuses dans l'estomac du Rat. Académie Royale des sciences et des lettres de Danemark. Extrait du Bulletin de l'année 1913, No. 1.

(2) Fibiger, Johannes: Untersuchungen über eine Nematode (Spiroptera sp. n.) und deren Fähigkeit, papillomatöse und carcinomatöse Geschwulstbildungen im Magen der Ratte hervorzurufen. Ztschr. f. Krebsforsch., 1913, xiii, 217.

(3) Fibiger, Johannes: Sur le développement de tumeurs papillomateuses et carcinomateuses dans l'estomac du rat sous l'action d'un ver nématode. Travaux de la troisième conférence internationale pour l'étude du cancer, 1913, Bruxelles, 1914.

(4) Fibiger, Johannes: Weitere Untersuchungen über das Spiropteracarcinom der Ratte. Ztschr. f. Krebsforsch., 1914, xiv, 295.

(5) Fibiger, Johannes and Ditlevsen H.: Contributions to the biology and morphology of spiroptera (Gongylonema) neoplastica n. sp. Mindeskrift for Japetus Steenstrup, 1914.

(6) Fibiger, Johannes: Undersögelser over Spiroptera neoplasticas Virkning paa Mus. 16'Skandinaviske Naturforskermöte i Kristiania 1916.

(7) Fibiger, Johannes: Ueber Disposition der Ratten und Maüse für die Wirkungen der Spiroptera neoplastica. Centralbl. f. allg. Path. u. path. Anat., 1916, xxvii.

(8) Fibiger, Johannes: Investigations on the Spiroptera Cancer, III. On the transmission of Spiroptera neoplastica (Gongylonema n.) to the rat as a method of producing cancer experimentally. Det kongelige Danske Videnskabernes Selskab (Académie Royale des sciences et des lettres de Danemark). Biologiske Meddelelser I_9., 1918.

(9) Fibiger, Johannes: Investigations on the Spiroptera cancer, IV. Spiroptera cancer of the tongue in rats. Det kongelige Danske Videnskabernes Selskab. Biologiske Meddelelser I_{10}., 1918.

(10) Fibiger, Johannes: Investigations on the Spiroptera cancer, V. On the growth of small carcinomata and on predisposition to Spiroptera cancer in rats and mice. Det kongelige Danske Videnskabernes Selskab. Biologiske Meddelelser I_{11}., 1918.

(11) Fibiger, Johannes: Investigations on the Spiroptera cancer, VI. A transplantable Spiroptera carcinoma of the mouse. Det kongelige Danske Videnskabernes Selskab. Biologiske Meddelelser I_{14}., 1919.

(12) Waetzold: Ztschr. f. Krebsforsch., 1919.

INDEX